TO STUDY ALLOIMMUNIZATION AND AUTOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA MAJOR PATIENTS

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I hereby declare that the thesis entitled "**TO STUDY ALLOIMMUNIZATION AND AUTOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA MAJOR PATIENTS**" submitted towards partial fulfillment of the requirements for the award of **MD in IMMUNOHEMATOLOGY AND BLOOD TRANSFUSION** degree to BFUHS is a compilation of original work carried out by me under the supervision of **Dr Rajesh Kumar**, Professor, Department of IHBT, **Dr. Amarjit Kaur**, Professor & Head, Department of IHBT and **Dr. Sonia Gupta,** Associate Professor, Department of IHBT. No part of this thesis has formed the basis for the award of any degree previously.

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Dedicated

to

My Grand

Parents

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ABBREVIATIONS

AHG	-	Anti- Human Globulin
AHTR	-	Acute Hemolytic Transfusion Reaction
CAT	-	Column Agglutination Technique
CMV	-	Cytomegalo Virus
CTT	-	Conventional Tube Technique
DAT	-	Direct Agglutination Test.
DCT	-	Direct Coomb's Test
DHTR	-	Delayed Hemolytic Transfusion Reaction
EDTA	-	Ethylene Diamine Tetra Acetic Acid
FUT	-	Fucosyl Transferase
FY	-	Duffy
Hb	-	Hemoglobin
HbA	-	Hemoglobin A
HDFN	-	Hemolytic Disease of Fetus and Newborn
HLA	-	Human Leucocyte Antigen.
HPC	-	Hematopoietic Progenitor Cells
HPSC	-	Hemopoietic Stem Cell Transplant
IAT	-	Indirect Agglutination Test
ID-MTS	-	ID Microtyping System
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
ISBT	-	International Society of Blood Transfusion
JK	-	Kidd
KEL	-	Kell
LE	-	Lewis
LISS	-	Low Ionic Strength Solution
LKE	-	Luke
Lu	-	Lutheran
MCH	-	Mean Corpuscular Hemoglobin
MCV	-	Mean Corpuscular Volume
No.	-	Number
NTDT	-	Non Transfusion Dependent Thalassemia

PEG	-	Poly Ethylene Glycol
RBC	-	Red Blood Cell
rpm	-	Rotations per minute
Se	-	Secretor
SPRCA	-	Solid Phase Red Cell Adherence

ABSTRACT

Introduction: Beta thalassemia is characterized by major or total suppression of beta chain synthesis. Lifelong transfusion every 2-4 weeks is required to maintain hemoglobin levels. The development of RBC antibodies both alloantibodies and autoantibodies can significantly complicate the transfusion therapy which further can cause hemolytic type of transfusion reaction.

Aims & Objectives: The aim of the present study was to determine the frequency of alloimmunization and autoimmunization among thalassemia major patients.

Material & Methods: This prospective study was carried out on all the thalassemia major patients registered with the hospital. Clinical and transfusion records of all the patients were examined for age of patients, age at initiation of transfusion therapy, total number of blood units transfused, transfusion interval, status of splenectomy, or other interventions. Alloantibody screening and identification was done using 3-cell (Bio-rad). If 3 cell panel was positive, 11 cell Antibody Identification-panel (Bio-rad, Switzerland) was used. To detect autoantibodies, auto-control was performed by DCT and Auto-Control using polyspecific (IgG+c3d) gel cards.

Results- 18 out of 200 patients developed alloantibodies. Most prevalent was Anti-Kell (50%), followed by Anti-E (4%), Anti Jk^a(4.5), Anti-Le^a (4.5%), Anti- Le^b (4.5%), Anti- C^w (4.5%), Anti-Kp^a(4.5%), Anti-c (4.5%) and Anti-C (4.5%). Out of 22 antibodies 31% antibodies belonged to Rh Blood group system and 54.5% antibodies were from Kell Blood group system. Age at first transfusion was higher in alloimmunized patients (p=0.002).27 out of 200 patients had autoantibodies. 6 patients had both allo and autoantibodies. 3 of the alloimmunized patients had splenectomy done.

Conclusion- The rate of alloimmunization was 9% amongst transfusion dependent thalassemia major patients. Out of 22 antibodies 31% antibodies belonged to Rh Blood group system and 54.5% antibodies were from Kell Blood group system. Antigen matched (at least for Rh and Kell) is the need of the hour to prevent alloimmunization.

Keywords: Alloimmunization, Autoimmunization, Beta thalassemia major, transfusion.

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INTRODUCTION

The thalassemias are the most common monogenetic diseases worldwide [1]. Thalassemias are inherited disorders caused by mutations in globin genes that decrease the synthesis of α- or β-globin. Decreased synthesis of one globin results not only in a deficiency of Hb, but also in red cell damage that is caused by precipitates formed from excess unpaired “normal” globin chains. As a result, there is an overall deficit of hemoglobin tetramers in the red blood cells (RBC) and the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are reduced[2].

The mutations that causes thalassemia are particularly common in Mediterranean, African and Asian regions. Thalassemia is most prevalent in Asians because of large population movement across countries. But there is very limited data on prevalence of RBC antigens amongst Asians. In India, it is estimated that around 8000–10000 new thalassemics (homozygous) are born every year and beta thalassemia gene is found more commonly in Punjabis, Sindhis, Bengalis, and Gujaratis[3]. Recent gene analysis have established that beta-thalassemia may arise from any of between 40-50 different mutations of beta globin chain.

Defective synthesis of beta-globin chain in beta- thalassemia contributes to anaemia through two mechanisms: (1) inadequate HbA formation, resulting in small (microcytic), poorly hemoglobinized (hypochromic) red cells and (2) by allowing the accumulation of unpaired alpha-globin chains which forms the toxic precipitates that severely damage the membrane of red cells and erythroid precursors.

The β thalassemias can be classified into four general categories: silent carrier, trait/thalassemia minor, thalassemia intermedia, and thalassemia major. β thalassemia carriers have no hematologic abnormalities but do have elevated hemoglobin A2 on hemoglobin electrophoresis. Those with β thalassemia trait (β thalassemia minor) have elevated A2 as well as mild anemia and microcytosis. They do

not require red cell transfusion. β thalassemia intermedia presents late with variable levels of anemia and is of the Non-transfusion-dependent-thalassemia (NTDT) phenotype.

In the absence of stem cell transplantation (HPSC), the disease is treated by life-long red blood cell (RBC) transfusion [4] to keep the hemoglobin (Hb) level between 9 and 11.5 g/dL. Although repeated blood transfusion is a life saving for thalassemia patients, is associated with inherent risks of alloimmunization against red cells antigen. Alloimmunization is formation of antibodies against the foreign RBC antigens due to transfusion or pregnancy. Red blood cell (RBC) alloimmunization occurs due to genetic disparity between donor and recipient red cells antigens [5].

The development of alloantibodies and autoantibodies against RBC antigens causes laboratory difficulties during RBC crossmatching, shortens in vivo survival of transfused red cells, delays provision of safe transfusions, and may accelerate iron overloading [6,7]. Autoantibodies are the antibodies formed against patient's own RBC antigens which further shortens the in-vivo survival of RBC, causes clinical hemolysis and can cause problems in cross-matching. Patients with autoantibodies may have a higher transfusion rate and often require immunosuppressive drugs or splenectomy [8,9].

The factors for alloimmunization are complex and involve 3 main contributing elements: the RBC antigenic difference between donor and the recipient, the recipient immune status and immunomodulatory effect of the allogenic blood transfusion on the recipient's immune system[10].

Approaches for prevention of alloimmunization are under debate. They range from the provision of RBCs matched or all the major antigens associated with clinically significant antibodies to blood matched only for antibodies that have already been made. Alloimmunization further complicates the transfusion therapy due to difficulty in getting blood and delayed type of hemolytic transfusion reaction. Reasons for

controversy regarding following the best approach lay in the fact that many alloantibodies are not harmful and expensive prevention methods may therefore benefit only some patients.[11-13]

Not all antibodies causes hemolysis only the antibodies which are clinically significant can cause hemolysis. The term “clinically significant” in relation to alloantibodies refers to the antibodies which causes obvious hemolysis, or an antibody that does not cause overt hemolysis but is associated with laboratory signs of hemolysis or and antibody that an antibody that is not associated with any clinical or laboratory signs of hemolysis, but RBCs incompatible with it survive less than normal lifespan [14].

Common antigens causing alloimmunization are: D, E, e, C, c, K, k, Jk^a, Jk^b, Fy^a, Fy^b, S, s, M, N, Le^a, and Le^b. Clinically significant antibodies are IgG type and are capable of crossing placenta thus causing HDFN. IgG antibodies usually require 37°C incubation and react optimally at the antihuman globulin (AHG) phase of testing. IgM antibodies usually react optimally at ambient temperatures or below 22°C at the immediate spin phase of testing. Examples of IgM type of antibodies are A, B, H, I, M, N Le^a, Le^b, P1.

For thalassemia, the age of beginning transfusion influences the rate of alloimmunization, with children beginning transfusion before one year of age having less alloimmunization (11% vs. 30%).[15] There is limited data on the RBC phenotypes and the extent of alloimmunization among Asians.

AIMS AND OBJECTIVES

The aim of this study is

1. To study the frequency of RBC alloimmunization and autoimmunization among thalassemia patients.
2. To analyze the factors responsible for development of antibodies.

REVIEW OF LITERATURE

Karl Landsteiner opened the doors of blood banking by discovering the first ever human blood group system. In 1901, Landsteiner drew blood from himself and five associates, separated the cells and serum, and then mixed each cell sample with each serum.[16] He was the first individual to perform forward and reverse grouping. ABO grouping is the most common blood grouping performed in blood banking. Both ABO forward and reverse grouping tests must be performed on all donors and patients.[17] The theory of inheritance of ABO genes was first described in 1924. ABO genes follow Mendelian genetics and shows codominant expression.[18] There is always an inverse reciprocal relationship between the forward and reverse type; thus, one serves as a check on the other.

ABO BLOOD GROUP SYSTEM

The ABO antibodies are predominantly IgM, activate complement, and react at room temperature or colder..[19] It has been postulated that bacteria, pollen particles, and other substances present in nature are chemically similar to A and B antigens. All other defined blood group systems do not regularly have “naturally occurring” antibodies expected in their serum to antigens they lack on their RBCs.

Antibody production in most other blood group systems requires the introduction of foreign RBCs by either transfusion or pregnancy, although some individuals can occasionally have antibodies present that are not related to the introduction of foreign RBCs. One position, or locus, on each chromosome 9 is occupied by an A, B, or O gene.[20]

Rh BLOOD GROUP SYSTEM

Rh blood group system is another very important and complex system. Alloimmunization with Rh Blood group antigens can complicate transfusion and pregnancy. D antigen is the most immunogenic amongst all minor blood group antigens. The term Rh refers to a specific red blood cell (RBC) antigen (D) and to a complex blood group system currently composed of 61 different antigenic specific.

There are four terminologies used to describe the Rh system. Two are based on postulated genetic theories of Rh inheritance. The third common terminology used describes only the presence or absence of a given antigen. The fourth was established by the International Society of Blood Transfusion (ISBT) Committee on Terminology for Red Cell Surface Antigens. RHD and RHCE are two closely linked genes located on chromosome 1 that control expression of Rh proteins.[21]

RHD codes for the presence or absence of the RhD protein, and the second gene RHCE codes for either RhCe, RhcE, Rhce, or RhCE proteins. Another gene important to Rh antigen expression is RHAG, and it resides on chromosome 6.[22] Rh antigens The antigens are found only on RBCs, and are not soluble or expressed on other cells.

The Rh antigens are well developed at birth, and as such can cause hemolytic disease of the fetus and newborn (HDFN). Rh antigens are also immunogenic, and exposure to foreign antigens through transfusion or pregnancy can cause an immune response with the production of corresponding antibodies. The five common Rh antigens are D, C, E, c, and e, and their specific corresponding antibodies account for the majority of problems due to Rh antibodies.

Rh antibodies are IgG immunoglobulins and react optimally at 37°C or after antiglobulin testing in any method used for antibody detection. Rh antibodies are usually produced following exposure of the individual's immune system to foreign RBCs, through either transfusion or pregnancy. Rh antibodies may show dosage, reacting preferentially with RBCs possessing double-dose Rh antigen.

Most commonly found Rh antibodies are considered clinically significant. Therefore, antigen-negative blood must be provided to any patient with a history of Rh antibody sensitization, whether the antibody is currently demonstrable or not. Rh antibodies do not bind complement.

LEWIS BLOOD GROUP SYSTEM

The Lewis blood group system is unique because the Lewis antigens are not intrinsic to RBCs but are on type 1 glycosphingolipids that are passively adsorbed onto the RBC membrane from the plasma. The Lewis system was named after one of the first individuals to make the antibody, reported by Mourant in 1946.[23]

There are several Lewis antigens, but the two of primary concern are Lea and Leb. The Lewis (Le, FUT3) gene is located on chromosome 19 at position 19p13.3, as is the secretor (Se, FUT2) gene at 19q13.3.[24] Lewis antibodies are often naturally occurring and made by Le(a–b–) persons; that is, they occur without any known RBC stimulus.

They are generally IgM and do not cross the placenta. Anti-Lea is the most commonly encountered of the Lewis antibodies and is often detected in room temperature tests, but it sometimes reacts at 37°C and in the indirect antiglobulin test. Anti-Leb is not as common or generally as strong as anti-Lea. It is usually an IgM agglutinin and can bind complement.

P BLOOD GROUP SYSTEM

The P blood group system was introduced in 1927 by Landsteiner and Levine. The P blood group comprised the P, P1, and Pk antigens and, later, Luke (LKE), PX2, and NOR. The antibodies to P blood group system can clinically insignificant or they can cause potential hemolysis. Anti-P1 is a common, naturally occurring IgM antibody in the sera of P1– individuals. Anti-P1 is typically a weak, cold-reactive saline agglutinin optimally reactive at 4°C and not seen in routine testing. Some examples react at room temperature, rarely they also react at 37’ and can bind complement, which can be detected by using polyspecific AHG cards. Anti-PP1Pk is produced by p individuals early in life without RBC sensitization and reacts with all RBCs except those of the p phenotype. The antibody is also associated with an increased incidence of spontaneous abortions in early pregnancy.

MNS BLOOD GROUP SYSTEM

In 1927 antibodies to M and N were discovered from rabbit sera. Walsh and Montgomery discovered S, its antithetical partner, s, was discovered in 1951. Forty-nine antigens have been included in the MNS system, making it almost equal to Rh in size and complexity. In 1953, an antibody to a high-prevalence antigen, U (for almost universal distribution), was named by Weiner. The observation by Greenwalt and colleagues [25] that all U– RBCs were also S–s– resulted in the inclusion of U into the system.

Many examples of anti-M are naturally occurring saline agglutinins that react below 37°C. Although we may think of agglutinating anti-M as IgM, 50% to 80% are IgG or have an IgG component.[26] Anti- M is known to show dosage phenomenon. Anti-N is not clinically significant unless it reacts at 37°C. It has been implicated only with rare cases of mild HDFN. Anti-N is less common than anti-M. Anti-N was also seen in renal patients, regardless of their MN type, who were dialyzed on equipment sterilized with formaldehyde. Most examples of anti-S and anti-s are IgG, reactive at 37°C and the antiglobulin phase of testing.

A few express optimal reactivity between 10°C and 22°C by saline indirect antiglobulin test. If anti-S or anti-s specificity is suspected but the pattern of reactivity is not clear, incubating tests at room temperature and immediately performing the antiglobulin test (without incubating at 37°C) may help in identification.

KELL BLOOD GROUP SYSTEM

The Kell blood group system consists of 36 high-prevalence and low-prevalence antigens; it was the first blood group system discovered after the introduction of antiglobulin testing. Anti-K was identified in 1946 in the serum of Mrs. Kelleher. The antibody reacted with the RBCs of her newborn infant, her older daughter, her husband, and about 7% of the random population. In 1949, anti-k, the high-prevalence antithetical partner to K, was described. Kp^a and Kp^b were described in 1957 and 1958, respectively. Likewise, Js^a (described in

1958) and Js^b (described in 1963) were found to be antithetical and related to the Kell system. The 36 antigens included in the Kell blood group system, designated by the symbol KEL or 006 by the ISBT.

Kell blood group antigens are found only on RBCs. The associated Xk protein is found in erythroid tissues and in other tissues, such as brain, lymphoid organs, heart, and skeletal muscle.[27] The total number of K antigen sites per RBC is quite low: only 3,500 up to 18,000 sites per RBC.[28] Despite its lower quantity, K is very immunogenic. KEL is a type -2 membrane glycoprotein, it spans the membrane once and has a short N-terminal domain in the cytosol and large C-terminal domain outside the membrane. [29,30]

Outside of the ABO and Rh antibodies, anti-K is the most common antibody seen in the blood bank. Anti-K is usually IgG and reactive in the antiglobulin phase, but some examples agglutinate saline-suspended RBCs. The antibody is usually made in response to antigen exposure through pregnancy and transfusion and can persist for many years. Naturally occurring IgM examples of anti-K are rare and have been associated with bacterial infections. Anti-K has been implicated in severe HTRs.

Although some examples of anti-K bind complement, in vivo RBC destruction is usually extravascular via the macrophages in the spleen. Anti-K is also associated with severe HDFN. Fetal anemia in anti-K HDFN is associated with suppression of erythropoiesis due to destruction of erythroid precursor cells, which can be additional to destruction of circulating antigen-positive RBCs, as seen in anti-D HDFN. Kell glycoprotein is expressed on fetal RBCs at a much earlier stage of erythropoiesis than Rh antigens.[31]

Antibodies to the low-prevalence Kell antigens are rare because so few people are exposed to these antigens. Antibodies to high-prevalence Kell system antigens are rare, so few people lack these antigens. They also parallel anti-K in serologic characteristics and clinical significance. Kx is present on all RBCs except those of the rare McLeod phenotype.

DUFFY BLOOD GROUP SYSTEM

The Duffy blood group system was named for Mr. Duffy, a multiply transfused haemophiliac who in 1950 was found to have the first described example of anti-Fy^a. One year later, the antibody defining its antithetical antigen, Fy^b, was found in the serum of a woman who had had three pregnancies. The Duffy antigens most important in routine blood bank serology are Fy^a and Fy^b. The FY glycoprotein may belong to the junctional membrane protein complex, which also contains Rh proteins[32]

They can be identified on fetal RBCs as early as 6 weeks gestational age and are well developed at birth. The antigens have not been found on platelets, lymphocytes, monocytes, or granulocytes, but they have been identified in other body tissues, including brain, colon, endothelium, lung, spleen, thyroid, thymus, and kidney cells.[29] Fy^a and Fy^b are destroyed by common proteolytic enzymes used in routine blood banking.

Anti-Fy^a is a common antibody and is found as a single specificity or in a mixture of antibodies. Anti-Fy^a occurs three times less frequently than anti-K. Anti-Fy^b is 20 times less common than anti-Fy^a and often occurs in combination with other antibodies. The antibodies are usually IgG and react best at the antiglobulin phase.

Rare examples of anti-Fy^a and anti-Fy^b bind complement. Some examples of anti-Fy^a and anti-Fy^b show dosage, reacting more strongly with RBCs that have a double dose than RBCs from heterozygotes. Anti-Fy^a and anti-Fy^b have been known to cause acute and delayed type Hemolytic Transfusion Reactions and can also cause HDFN. Once the antibody is identified antigen negative blood must be issued to the patient.

KIDD BLOOD GROUP SYSTEM

In 1951, Allen and colleagues found Anti-Jk^a antibody in the serum of Mrs. Kidd. Its antithetical partner, Jk^b, was found 2 years later. The Kidd system is designated by the symbol JK or 009 by the ISBT. Jk^a and Jk^b are antigens commonly found on RBCs of most individuals. Jk^a

and Jk^b antigens are well developed on the RBCs of neonates. Jk^a has been detected on fetal RBCs as early as 11 weeks; Jk^b has been detected at 7 weeks. The Kidd antigens are not very immunogenic.

Kidd antibodies have a notorious reputation in the blood bank. They demonstrate dosage, are often weak, and are found in combination with other antibodies, all of which make them difficult to detect. Anti-Jk^a is more frequently encountered than anti-Jk^b. The antibodies are usually IgG (antiglobulin reactive) but may also be partly IgM and are made in response to pregnancy or transfusion. Antibody reactivity can also be enhanced by using LISS or PEG.

Many examples of the Kidd antibodies bind complement. Rare examples are detected only by the complement they bind. The titer of anti-Jk^a or anti-Jk^b quickly declines in vivo. The decline in antibody reactivity and the difficulty in detecting Kidd antibodies are reasons why they are a common cause of HTRs, especially of the delayed type(DHTR). most Kidd antibodies are only rarely associated with severe cases of HDFN.[33]

LUTHERAN BLOOD GROUP SYSTEM

In 1945, anti-Lua was found (and described in detail a year later) in the serum of a patient with lupus erythematosus, following the transfusion of a unit of blood carrying the corresponding low-prevalence antigen.[34] Twenty-four antigens are part of the Lutheran system. Most of these antigens are high prevalence; four sets of antigens are antithetical. Although the antigens have been detected on foetal RBCs as early as 10 to 12 weeks of gestation, they are poorly developed at birth. As a result, HDFN is rare and only mild.

Lutheran glycoprotein is widely distributed in tissues: brain, lung, pancreas, placenta, skeletal muscle, and hepatocytes (especially fetal hepatic epithelial cells). The presence of Lutheran glycoprotein on placental tissue may result in adsorption of maternal antibodies to Lutheran antigens, thus decreasing the likelihood of HDFN.

Lutheran antigens are resistant to the enzymes ficin and papain and to glycine-acid EDTA treatment but are destroyed by treatment with the enzymes trypsin and α-chymotrypsin. Lua and Lub are antigens produced by allelic codominant genes. The number of Lub sites per RBC is low, estimated to be from 1,640 to 4,070 on Lu(a–b+) RBCs and from 850 to 1,820 on Lu(a+b+) RBCs. Most examples of anti-Lua are IgM naturally occurring saline agglutinins that react better at room temperature than at 37°C. A few react at 37°C by indirect antiglobulin test.

Some are capable of binding complement, but in vitro hemolysis has not been reported. Anti-Lua is more likely encountered as an incompatible crossmatch or during an antibody workup for another specificity. There are no documented cases of immediate HTRs; there are only rare and mild delayed HTRs due to anti-Lua.

Although the first example of anti-Lub was a room temperature agglutinin, and IgM and IgA antibodies have been noted, most examples of anti-Lub are IgG and reactive at 37°C at the antiglobulin phase. The antibody is made in response to pregnancy or transfusion. Anti-Lub has been implicated with shortened survival of transfused cells and post-transfusion jaundice, but severe or acute hemolysis has not been reported.

Anti-Lu3 is a rare antibody that reacts with all RBCs except Lu (a–b–) RBCs. Only a few antibody specificities are commonly seen: antibodies to M, P1, and I react at room temperature and are considered clinically insignificant, antibodies to K, S, s, Fya, Fyb, Jka, and Jkb react in the antiglobulin phase and are clinically significant.

I AND i BLOOD GROUP SYSTEM

The I and I antigens are best introduced by classic serologic facts. Both I and i are high-prevalence antigens, but they are expressed in a reciprocal relationship that is developmentally regulated. At birth, infant RBCs are rich in i; I is almost undetectable. During the first 18 months of life, the quantity of i slowly decreases as I increases until adult

proportions are reached; adult RBCs are rich in I and have only trace amounts of i antigen. I and i are not antithetical antigens. Rather, they are branched and linear carbohydrate structures, respectively, that are formed by the action of glycosyl transferases.

The gene encoding the transferase that converts i active straight chains into I active branched chains has been cloned, and several mutations responsible for the rare adult i phenotype have been identified.[35] Both I and i are high-prevalence antigens. At birth, infant RBCs are rich in i; I is almost undetectable. During the first 18 months of life, the quantity of i slowly decreases as I increases until adult proportions are reached; adult RBCs are rich in I and have only trace amounts of i antigen. At birth, infant RBCs are rich in i; I is almost undetectable. During the first 18 months of life, the quantity of i slowly decreases as I increases until adult proportions are reached; adult RBCs are rich in I and have only trace amounts of i antigen.

Anti-I is a common autoantibody that can be found in virtually all sera, although testing at 4°C and/or against enzyme treated RBCs may be required to detect the reactivity.[36] Autoanti-I, found in the serum of many normal healthy individuals. It is usually a weak, naturally occurring, saline-reactive IgM agglutinin with a titer less than 64 at 4°C. Incubating tests in the cold enhances anti-I reactivity and helps confirm its identity; albumin and enzyme methods also enhance anti-I reactivity.

Patients with M. pneumoniae often develop strong cold agglutinins with I specificity and can experience a transient episode of acute abrupt hemolysis just as the infection begins to resolve. Alloanti-i has never been described. Most examples of autoanti-i are IgM and react best with saline suspended cells at 4°C. I and i have also been found in the plasma and serum of adults and newborns and in saliva, human milk, amniotic fluid, urine, and ovarian cyst fluid.

DIRECT AGGLUTINATION TEST

Before the antiglobulin test was developed, only IgM antibodies were detected by direct testing methods. Discovery of AHG helped in the

detection of nonagglutinating IgG antibodies. In 1946, Coombs and coworkers described the use of AHG to detect in vivo sensitization of the RBCs of neonates suffering from hemolytic disease of the fetus and newborn (HDFN). The test was originally called the Coombs' test but later was called the direct antiglobulin test (DAT).[37]

The antiglobulin test can be used to detect RBCs sensitized with IgG alloantibodies, IgG autoantibodies, and complement components. Sensitization can occur either in vivo or in vitro. The use of AHG to detect in vitro sensitization of RBCs is a two-stage technique referred to as the indirect antiglobulin test (IAT). In vivo sensitization is detected by a one-stage procedure called the direct antiglobulin test (DAT). The IAT and DAT still remain the most common procedures performed in blood group serology.

Anti-Human Globulin (AHG) reagents can be polyspecific or monospecific. Polyspecific AHG reagents contain antibody to human IgG and to the C3d component of human complement. Therefore, its use can facilitate agglutination when RBCs have been sensitized with IgG or C3d or both. Monospecific AHG reagents contain only one antibody specificity: either anti-IgG or antibody to specific components of complement, such as C3b or C3d (i.e., anticomplement).

Initial DATs include testing one drop of a 3% to 5% suspension of washed RBCs with polyspecific (anti-IgG, anti-C3d) reagent. Positive results are monitored by a DAT panel using monospecific anti-IgG and anti-C3d to determine the specific type of protein sensitizing the cell. The saline control serves to detect spontaneous agglutination of cells or reactions occurring without the addition of AHG reagents.

INDIRECT AGGLUTINATION TEST

The IAT is performed to determine in vitro sensitization of RBCs and is used in the following situations:

- Detection of incomplete (nonagglutinating) antibodies to potential donor RBCs (compatibility testing) or to screening cells (antibody screen) in serum

- Determination of RBC phenotype using known antisera (e.g., weak D, any other antigen testing that requires IAT)
- Titration of incomplete antibodies.

CONVENTIONAL TUBE TECHNIQUE

Only a small percentage of the general population (between 0.8% and 2%) has detectable RBC alloantibodies.[38] The AABB's Standards for Blood Banks and Transfusion Services requires the use of an antibody screen to detect clinically significant antibodies in allogeneic blood donors and in patients as part of pretransfusion compatibility testing for whole blood, RBCs, and granulocyte components.[39]

An antibody screen is also included in standard prenatal testing for obstetric patients to evaluate the risk of HDFN and to assess the mother's candidacy for Rh-immune globulin (RhIg) prophylaxis.[40] An antibody screen may additionally be performed when evaluating the compatibility of allogeneic hematopoietic progenitor cell (HPC) and bone marrow donors with the intended transplant recipient.[41]

Conventional tube technique (CTT) is the gold standard for detection of in vivo and in vitro sensitization of RBC. 5% suspension is used. Tube testing offers flexibility of test at different phases and the option to use a variety of additive solutions (and thus obtain varying degrees of sensitivity).It also requires little specialized equipment.

Commercial reagent red cells for tube testing are diluted to a 2% to 5% suspension in a preservative solution that can be used directly from the bottle. Washing the red cells before use is usually unnecessary unless the preservative solution is suspected of interfering with antibody identification. When tube tests are performed and reactivity occurs at several phases, each phase should be analysed separately. The pattern at room temperature may indicate a different specificity from the pattern at IAT phase.

COLUMN AGGLUTINATION TECHNOLOGY

Column Agglutination technology (CAT) uses gel particles to trap the agglutinates. It does not require multiple saline washes, as required in CTT. The gel test was developed in Europe by Yves Lapierre at DiaMed AG (Murten, Switzerland). Inc. In March 2002, Ortho Clinical Diagnostics, Inc. acquired MTS, and its gel-based test has been named the ID-Micro Typing System (ID-MTS).

The gel test, which is performed in a specially designed microtube, is a hemagglutination test based on controlled centrifugation of RBCs through a dextran-acrylamide gel that contains predispensed reagents. Each microtube is composed of an upper reaction chamber that is wider than the tube itself and a long, narrow portion referred to as the column.

In the gel test, a plastic card with microtubes is used instead of test tubes. A gel card measures approximately 5 × 7 cm and consists of six or eight columns. Each microtube contains predispensed gel, diluent, and reagents, if applicable. The reaction chamber is where RBCs may be sensitized (antigen-antibody binding) during incubation.

The column of each microtube contains dextran-acrylamide gel particles suspended in a diluent. The gel particles make up 75% of the gel-liquid mixture. The gel particles are porous, and they serve as a reaction medium and filter, sieving the RBC agglutinates according to size during centrifugation. Large agglutinates are trapped at the top of the gel and are not allowed to travel through the gel during the centrifugation of the card. Agglutinated RBCs remain trapped in the gel, while unagglutinated RBCs travel unimpeded through the length of the microtube, forming a pellet at the bottom following centrifugation.

Agglutination reactions in the gel test are graded from 1+ to 4+ and mixed-field, A 4+ reaction is characterized by a solid band of agglutinated RBCs at the top of the column. 3+ reaction, most of the agglutinated RBCs remain near the top of the column, with a few agglutinates staggered below the thicker band, majority of agglutinates

are observed in the top half of the column. In a 2+ reaction and In a 1+ reaction, RBC agglutinates are predominantly seen in the lower half of the column, with some RBCs at the bottom of the microtube.

ADVANTAGES OF CAT

1. Standardization is one of the major advantages
2. stable, well-defined endpoints of the agglutination reaction that can be observed or reviewed for up to 3 days.
3. no wash step, and no need for antiglobulin control cells.

DISADVANTAGES OF CAT

1. Sample restrictions
2. Need for special equipment.
3. Hemolyzed or grossly icteric blood samples should not be used because the results may be difficult to interpret visually.
4. Grossly lipemic samples containing particulates may clog the column
5. Rouleaux caused by serum or plasma with abnormally high concentrations of protein may produce hazy reactions or false-positive results.

SOLID PHASE TECHNOLOGY

In 1986, Beck and co-workers reported using SPRCA to detect RBC antigens and antibodies.[42] SPRCA assays are available in two formats. In one format, the target antigen (RBCs, platelets, platelet proteins, etc.) is added by the user to the microplate wells before starting the test. Capture-R Select, for the identification of RBC anti - bodies, and Capture-P, for the detection of platelet antibodies, are examples of this format. In the other format, the target antigen (RBC stroma, platelet glycoproteins, or CMV) is bound to the microplate test wells during the manufacturing process. Capture-R Ready-Screen/Capture-R Ready-ID and Capture-P Ready-Screen are examples of this format.

Immucor SPRCA assays may be performed with either plasma or serum, but plasma is preferable. When clotted samples are

incompletely clotted, the serum is difficult to remove during the wash cycle; allowing residual unbound serum to clot and make the endpoint of the test unreadable.

To perform Immucor SPRCA testing, patient serum or plasma and low-ionic-strength saline (LISS) are added to microwells that are coated with the target antigen. The microplates are incubated at 37°C, allowing time for possible antibodies to attach to the antigens in the well. After incubation, the wells are washed with pH-buffered isotonic saline to remove unbound serum proteins. Next, anti-IgG–coated indicator RBCs are added, and the microwells are centrifuged. Centrifugation forces the indicator RBCs into close contact with IgG antibodies from the test serum/plasma that are bound to the immobilized target antigen, that is, the sandwich technique. If antibody is attached to the antigen during the incubation phase, the indicator cells form a monolayer of RBCs. If no antibody is present, nothing is attached to the antigen, and the indicator cells form a clearly delineated button at the center of the microplate well during centrifugation.

ADVANTAGES

1. Standardization
2. SPRCA provides stable, well-defined endpoints
3. No predilution of reagents is required, and it is possible to test hemolyzed, lipemic, or icteric samples
4. LISS in the test process provides enhanced sensitivity for detecting weak antibodies. Also, the LISS used in the Immucor Capture technology changes color when serum/plasma is added, a safety feature that ensures the patient sample was added to the test system when manual testing is performed.
5. because the reagent red blood cells in SPRCA are dried and precoated onto the surface of the wells, the test plates have a long shelf life.

DISADVANTAGES

1. Specialized equipment is required
2. The increased sensitivity may also be a disadvantage inasmuch as SPRCA may detect weak autoantibodies that other systems miss.

AUTOMATION

Full automation of the Capture solid-phase technology is available from Immucor, Inc., using two FDA-approved, walk-away instruments. The smaller instrument, Echo, can be continuously loaded and unloaded. It holds 20 samples, 16 reagents, and 32 microstrips. The larger instrument, NEO, is also fully automated. Both Echo and NEO use hemagglutination to perform blood typing and Capture assays to perfom antibody screening, antibody identification, weak D testing, IgG autologous control, and compatibility testing.

BIO-RAD SOLID-PHASE PROTEIN A TECHNOLOGY

In 1992, Biotest AG developed a test in Europe for detecting IgG antibodies using microplate wells that are coated with protein A. Protein A is a component of the cell wall of Staphylococcus aureus that has a very high affinity for the Fc portion of most immunoglobulin classes.[43]

The Erytype S blood group system is a hemagglutination microplate method that uses microplate wells precoated with blood-grouping monoclonal antibodies. Agglutination reaction results, and the agglutination patterns are captured and interpreted by the TANGO infinity[44] automation platform. Assays include forward and reverse ABO group, Rh type, and K (Kell) phenotype.

The Solidscreen II assay provides a standardized method for antiglobulin testing[45]. The assay combines traditional low-ionic-strength test methods with a unique solid-phase format. Unlike other solid-phase methods that use red blood cell stroma, the Solid screen II assays use a microwell coated with protein A, which has a high affinity for the Fc portion of IgG. The system uses the same liquid reagent red

blood cells and low-ionic-strength solution used in tube methods for antibody screening and identification.

In a study performed by Singer et al[7] in 2000, clinical and transfusion records of 64 patients were analyzed 19 alloantibodies were identifies, 14 of them clinically significant. 3 patients developed 2 antibodies, and 3 antibodies were detected in 1 patient. Out of 64 patients 6 patients developed autoantibodies as determined by a persistent or transient positive DAT that ranged between +1 to +4 and was based on a maximum score of 4+. In 7 patients autoantibodies were associated with alloantibodies.

In another study performed by Khalid et al[46] (in 2004) 75 cases of multiply transfused thalassemia major patients were investigated for development of alloantibodies against red cells by indirect antiglobulin test, using 3-red cell panel, and when required 11-red cell panel. Anti-red cell alloantibodies were detected in 17 (22.7%) patients. Anti-Kpa antibodies were the commonest, followed by Anti-e, anti-E and anti-K antibodies, respectively. Anti-k, -C^{W}, -Fy^{b}, -Kpb, -Rh 'D' and –c were detected in one patient each.

A study conducted by Sadeghian et al. [47] in 2009, identified 12 alloantibodies in 9 patients (2.87%) that were all against Rhesus (Rh) blood group antigens(D,C,E) and followed by Anti-C, Anti-E. Three patients developed 2 antibodies. Higher frequency of alloimmunization was observed in females, Rh negative and splenectomized patients.

Study conducted on 116 thalassemic patients by Gupta et al. [48] in 2011, found 11 alloantibodies (9.48%). In four (36%) patients first transfusion was given before 6 months of age and in seven (64%) patients, first transfusion was given after two years of age. The interval between consecutive transfusions varied from 18 to 35 days. The most common antibody was Anti-E found in 4 (36.4%) patients, followed by Anti-K (three patients, 27.2%), Anti-Kp(a) (two patients, 18.2%) and Anti-C(w) (two patients, 18.2%). The interval from first transfusion to antibody development varied from 1.5 to 14 years. None of the eight

out of 116 patients, who underwent splenectomy showed any antibody development.

Pahuja et al. [49] conducted a study on 211 patients and concluded that the frequency of alloimmunization was 3.79% and that of autoimmunization was 0.47%. The alloantibodies identified were anti-E, anti-K, anti-D, anti-Kpa, anti-C^w, anti-c and anti-Jk^a. In the present study, no significant association was observed between splenectomy and the development of alloantibodies as well as between age at initiation of transfusion and alloimmunization.

In another study performed by HK Dhawan[50], Vijay Kumawat, Deepak Bansal, Satyam Arora[51] et al in 2014 study was carried out 319 transfusion dependent thalassemic patients, 18 patients developed alloantibodies. A total of 23 alloantibodies were identified in patient population. Out of 23 alloantibodies, 52.17% belonged to Rh blood group system(Anti-E=17%, Anti D=13%, Anti C=13%, Anti C^w =9%), 35 % belonged to Kell Blood group system, 9% of Kidd, 4% of Xg blood group system. 14 patients developed single antibody while 4 patients developed dual antibody.

In study conducted by Datta et al.[3] it was found that In between 2 and 10 years of age total patients were 216 and among them 3 cases of alloimmunization were detected (1.38%). In 11–20 years of age total 6 patients had alloantibodies among 173 patients (3.47%). In 21–30 years of age total patients were 81 and among them 15 had alloantibodies (18.52%). In 31–40 years of age among 30 patients 4 had alloimmunization (13.33%). So it was found that the rate of alloimmunization increases with the age of the patients' population and approximately 67.86% (19/28) of total cases of alloimmunization were detected in the age group of 21–40 years.

Another study conducted by Amal et al. [52] in 2020 concluded that Of the 200 patients in their study, 94 were males and 106 females, with the age range of 2–37 years. Alloantibodies were detected in 36 (18%) of the patients, while autoantibodies were detected in 33 (16.5%). The

dominant alloantibodies were directed against Kell (33%) and Rh (24.4%) groups. Alloimmunization had a significant relationship with treatment duration and the frequency of transfusion (P= 0.007, 0.001, respectively). The presence of autoantibodies was signifcantly related to age (P= 0.001), total number of transfused units (P= 0.000) and splenectomy (P= 0.000).

Waldi et al.[52] conducted a study on 18 alloantibodies were detected in 13 of 40 patients (32.5%), with an alloimmunization rate of 0.26 antibodies per 100 units transfused. Thirteen antibodies (72.2%) were directed against Rh (5 anti-D, 4 anti-C, 2 anti-E, 1 anti-e, 1 anti-V).

MATERIAL & METHODS

The study was carried out on 200 multiply transfused patients with β-thalassemia major registered with thalassemia clinic. Informed consent was obtained from patients or their parents. Clinical and transfusion records of all the patients were examined for age of patients, age at diagnosis and initiation of transfusion therapy, total number of blood units transfused, transfusion interval, status of splenectomy or other interventions.

A volume of 2mL blood was drawn in EDTA tube as well as Plain vial for ABO & Rh, Antibody screening, DAT, and Antibody Identification.

ABO & Rh GROUPING

ABO & Rh grouping was done by both Column Agglutination Technology (Diapanel, Bio-rad, Cressier sur Morat, Switzerland).and Conventional Tube Technique.

Conventional Tube Technique- 1ml of blood was collected in EDTA vial and was centrifuged at 3000rpm for 3minutes. Plasma was separated and RBC were washed with Normal Saline 3 times by centrifuging at 3000 rpm for 3 minutes. A total of 8 clean glass test tubes were taken and were labelled as Anti-A, Anti-B, Anti-AB, Anti- D for forward grouping and A- Cell, B- cell , O cell And Autocontrol for Reverse grouping.2-5% suspension was made by adding 25microlitre of packed cells to 500microlitre Normal Saline. 100 microlitre Commercially available Anti-A, Anti-B Anti-AB and Anti-D were added in their respective labelled test tubes. 50microlitre prepared suspension was added in each test tube.

For Reverse Grouping, 2-5% suspension of washed and pooled A cells, B cells, O cells were added in their respective labelled test tubes. 50microlitre of plasma was added in each test tube. For Autocontrol, patient's own plasma was added to RBC suspension of patients' own RBCs.

Tubes were incubated at room temperature for 15 minutes and then centrifuged at 1000rpm for 1minute. Tubes were then gently agitated and degree of agglutination was scored. Interpretation of results was as follows.

4+ =cell button remains in one clump.

3+ =cell buttons dislodge into several clumps.

2+ cell buttons dislodge into many small clumps of equal size.

1+ cell button dislodges into finely granular, but definite, small clumps.

0 Negative reaction-cell buttons dislodges into no visible clumps.

Column agglutination technology-

Principle- The gel test, is a hemagglutination test based on controlled centrifugation of RBCs through a dextran-acrylamide gel that contains predispensed reagents. Each microtube is composed of an upper reaction chamber that is wider than the tube itself and a long, narrow portion referred to as the column. The column of each microtube contains dextran-acrylamide gel particles with Anti- A, Anti-B, Anti-D, one empty serve as negative control and two microtubes with neutral gel serve for reverse grouping with A1 cells and B cells. The columns can also be embedded with AHG for IAT and DAT. The gel particles are porous, and they serve as a reaction medium and filter, sieving the RBC agglutinates according to size during centrifugation. Agglutinated RBCs remain trapped in the gel, while unagglutinated RBCs travel unimpeded through the length of the microtube, forming a pellet at the bottom following centrifugation. The results are read as follows

4+= solid band of agglutinated RBCs at the top of the column.

3+= remain near the top of the column, with a few agglutinates staggered below the thicker band.

2+=RBC agglutinates are distributed throughout the upper and lower halves of the column, with a few agglutinates at the bottom of the microtube.

1+=RBC agglutinates are predominantly seen in the lower half of the column, with some RBCs at the bottom of the microtube.

0= the RBCs form a well-delineated pellet at the bottom of the microtube.

Procedure- A 2-5% cell suspension was made and 10 microlitre of the suspension was put in Anti-a, anti-B, anti-D and control column. 50microlitre of 0.8-1% suspension of pooled and washed A and B cells were put in their respective columns. 50microlitre of patient's plasma was put in reverse grouping columns. The card was incubated at room temperature and then centrifuged at 1050rpm for 10 minutes and the results were interpreted.

DIRECT AGGLUTINATION TEST

DAT was performed by Column Agglutination Technology

Method- 1ml of whole blood was collected in EDTA vial and was centrifuged at 3000rpm for 3 mins. A 0.8-1% suspension was prepared by adding 10microlitre packed cells in 1000microlitre of LISS. 50microlitre of this suspension was added in the reaction chamber of Bio-Rad LISS-Coomb's card which is embedded with AHG (IgG+C3d). The card was then centrifuged at 1000rpm for 10 mins and results were read.

INDIRECT AGGLUTINATION TEST

IAT was performed by Column Agglutination Test.

Method- 2ml of whole blood was collected in plain vial and the blood was allowed to clot. The vial was then centrifuged at 3000rpm for 3 minutes. Pooled O- cells were prepared by pooling phenotype (Rh & Kell) group O cells from donors. The cells were then washed three times with NS, each time rotation of 3000rpm was given for 3 minutes. A 0.8-1% suspension of these pooled was prepared by adding 10microlire of these cells to 1000microlitre of LISS. 50 microlitre of this suspension was added to AHG card (Bio-Rad) in reaction chamber

along with 25 microlitre of patient's serum. The card was then incubated at 37^{o} C for 15 minutes and was then centrifuged at 1050rpm for 10minutes and results were interpreted as explained above.

ANTIBODY SCREENING

Samples which tested positive for IAT were screened for antibodies using commercially available Bio-Rad 3- cell panels and CAT.

ANTIBODY IDENTIFICATION

Antibody specificity detection was performed using a commercial 11 cell identification panel (Diapanel, Bio-rad, Cressier sur Morat, Switzerland).

Antibody confirmation was done by phenotyping and using select cells.

PHENOTYPING

Phenotyping was done by using CAT.

Method- 2-5% suspension was prepared using by adding 25 microlitre of packed cells to 500 microlitre of LISS. 10 microlitre of suspension was added to each chamber of Rh and Kell phenotype cards. The card was then centrifuged at 1050rpm for 10minutes, and results were interpreted.

SELECT CELLS

Selected cells are chosen from other panel or screening cells to confirm or eliminate the antibody. The cells are "selected" from other panels because of their characteristics

- The number of selected cells needed depends on how many antibodies are identified.
- Every cell should be positive for each of the antibodies and negative for the remaining antibodies.

AUTOANTIBODIES

Autoantibodies were detected by incubating patient's own cell with patient's plasma at 37°C for 15 min and then centrifuging for 10 min on gel card containing polyspecific antihuman globulin (anti IgG + C3d)

INCLUSION CRITERIA

1. All thalassemia patients.

EXCLUSION CRITERIA

1. Alloimmunized Thalassemia major patients.

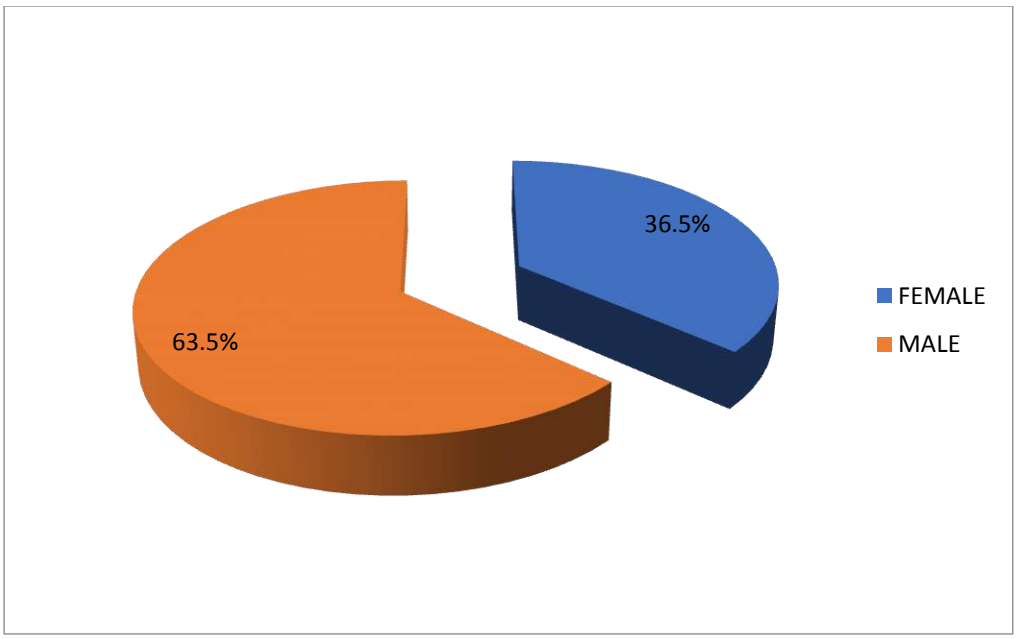
OUTCOME: Alloimmune and autoimmune antibodies in transfusion dependent thalassemia major patients.

STATISTICAL ANALYSIS: Analysis was conducted using SPSS for windows (version 21) by making frequency distribution tables and identifying frequency of alloimmunization and autoimmunization as well as specificity of the particular antibodies. Comparisons of categorical data will be made using Pearson Chi Square test and Fisher's exact test. A Mann-Whitney test was used for comparison of continuous variable. A p-value less than 0.05 will be considered significant.

OBSERVATIONS & RESULTS

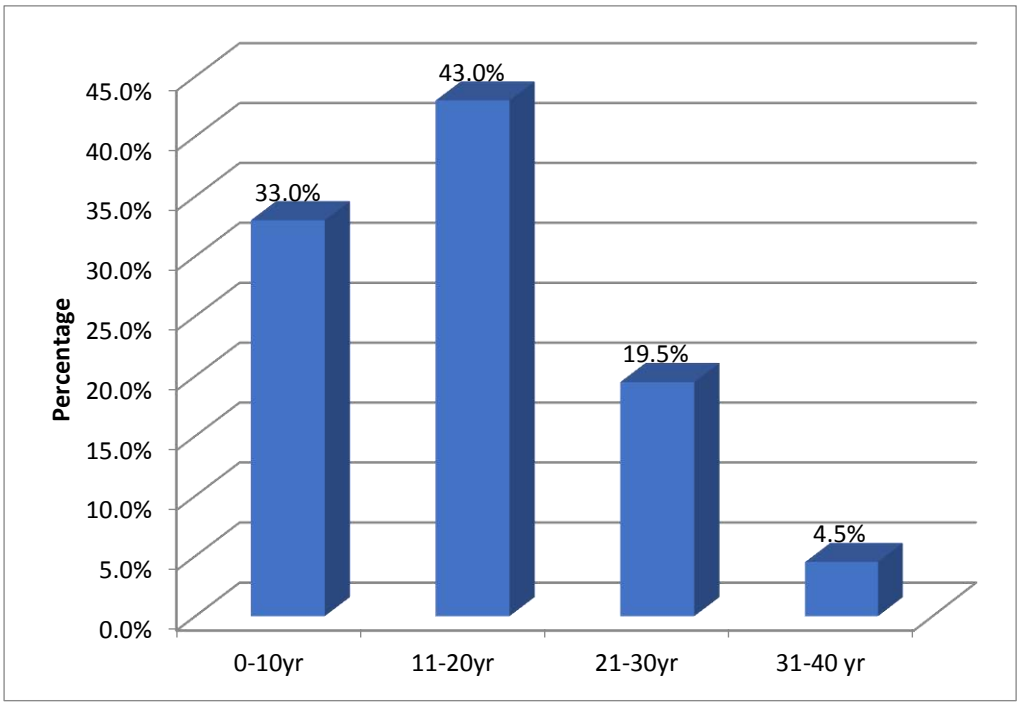
This study was carried out for a period of 1 year. 200 patients were evaluated during this period of time. The main aim of this study was to study alloimunization and autioimmuiation in thalassemia patients who are dependent of transfusion. Complete history of patients was taken with respect to age, sex, marital status, caste,address , age at first transfusion and total units transfused etc. Serological investigations like ABO and Rh were carried out using CTT and CAT and DAT, IAT , Antibody screen and Identification were carried out using CAT (Bio-Rad) and the observations are as follows.

FIG 1. DISTRIBUTION OF MALES AND FEMALES THALASSEMIA PATIENTS



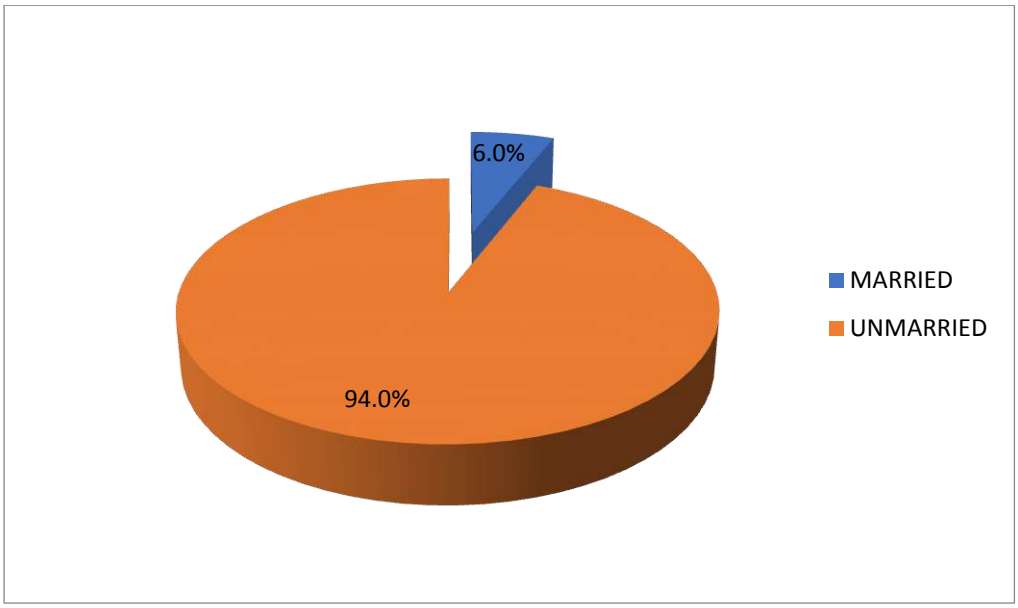
The study included 200 transfusion dependent Thalassemia major patients. Out of which 73 were females and 167 were males as shown in fig1.

FIG. 2: AGE DISTRIBUTION PATTERN AMONG THALASSEMIA PATIENTS.



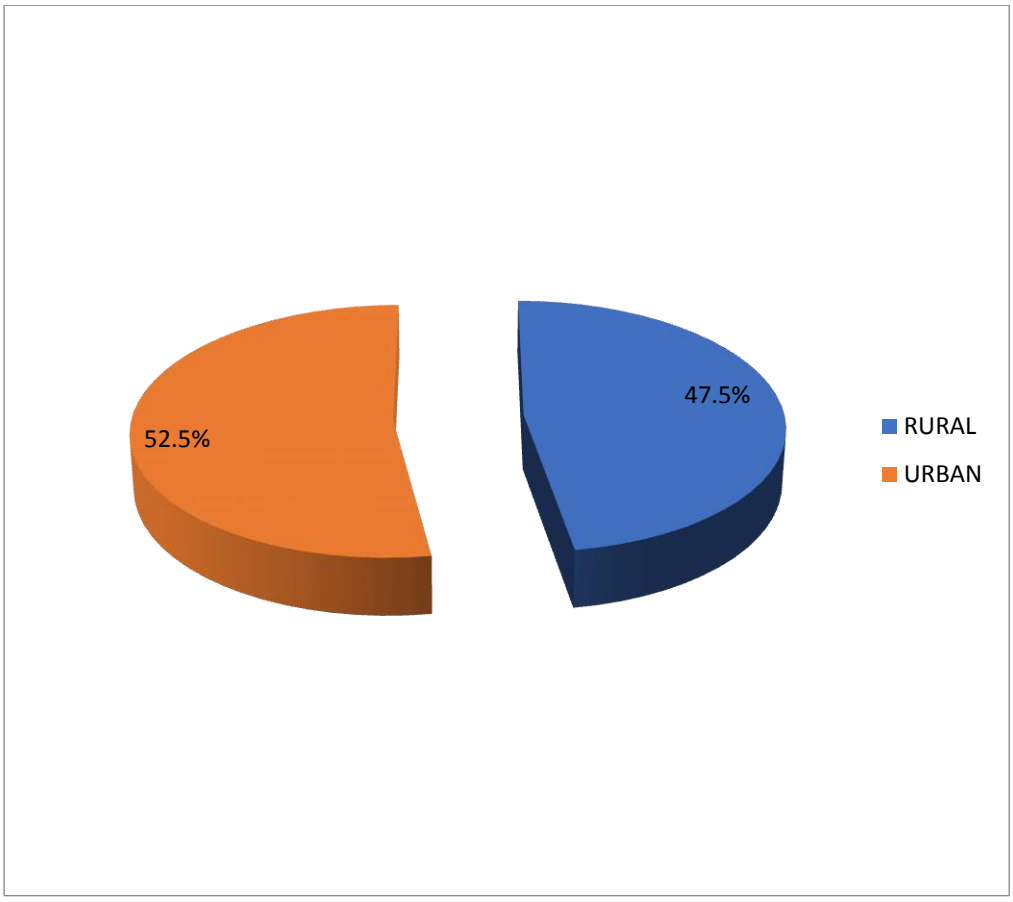
According to age distribution, patients were divided into 4 groups. Between the age group of 0-10yrs there were 66 patients, 86 patient lied in the age group of 11-20years, 39 patients were there in age group of 21-30years and 9 patients were present in the age group of 31-40years as shown in fig 2.

FIG 3: DISTRIBUTION OF MARITAL STATUS AMONGST PATIENTS



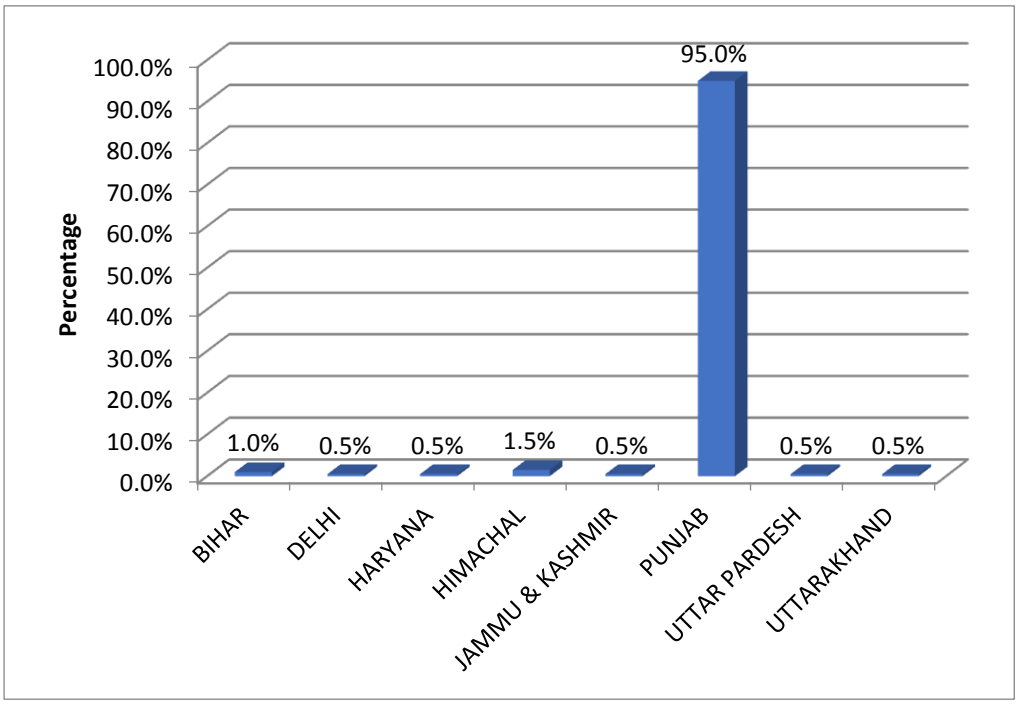
Upon taking complete history of the patients, it was found that 12 patients were married and rest of 188 patients were not married as shown in fig3.

FIG 4: DISTRIBUTION OF CASES IN RURAL AND URBAN AREAS.



It was found that cases of thalassemia cases were more prevalent in urban population as compared to the rural areas. Amongst the patients 105 belonged to urban areas whereas 95 belonged to rural areas as depicted in fig 4.

FIG 5: STATE WISE DISTRIBUTION OF PATIENTS ADMITTED IN OUR CENTER

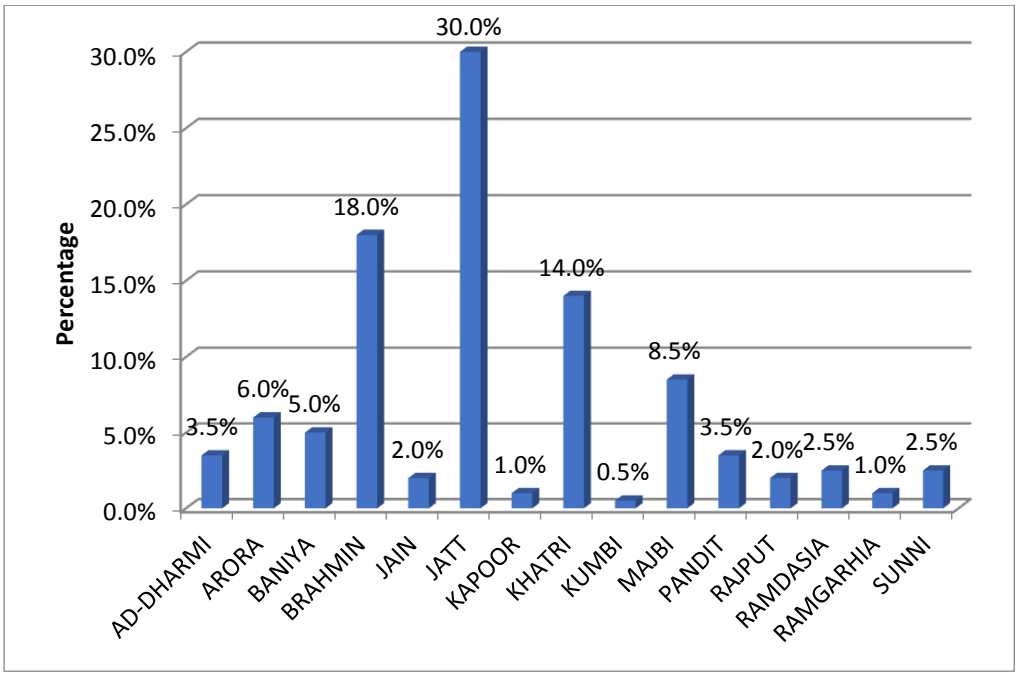


Among patients admitted in our centre 190 patients were from Punjab, 3 were from Himachal Pradesh, 2 from Bihar, 1 from Delhi , 1 from Uttar Pradesh,1 from Haryana, 1 from Uttarakhand and 1 from Jammu and Kashmir as shown in fig 5.

TABLE 1. CASTE BASED DISTRIBUTION OF PATIENTS

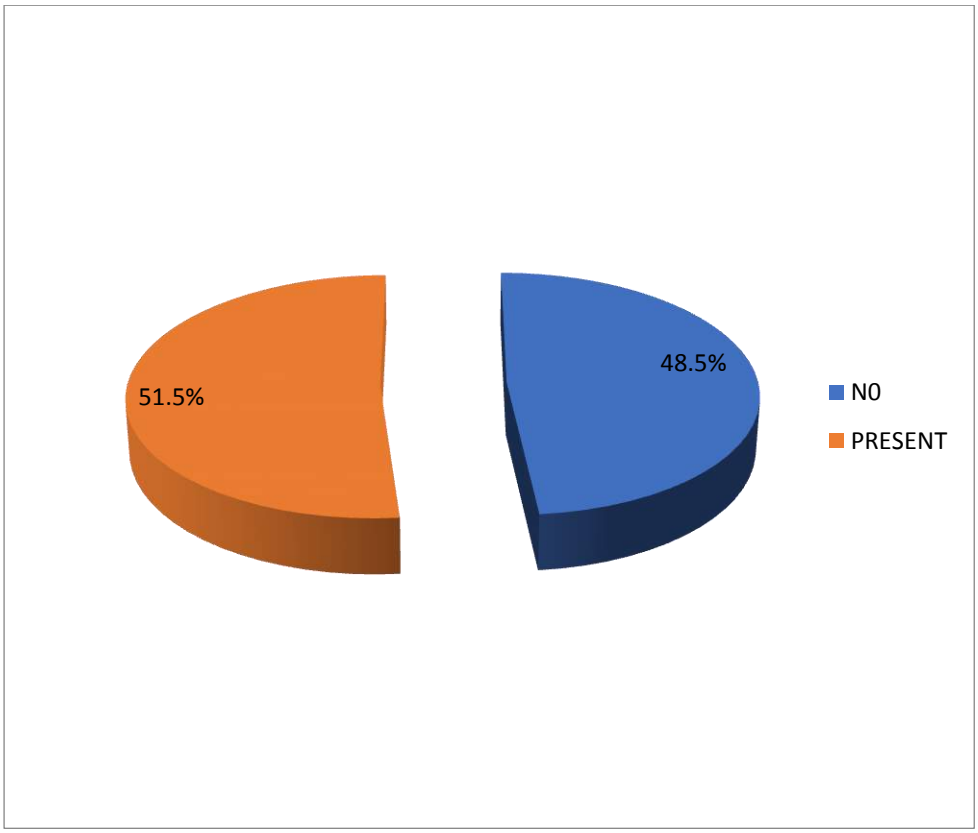
CASTE	NO. OF CASES	PERCENTAGE
AD-DHARMI	7	3.5%
ARORA	12	6.0%
BANIYA	10	5.0%
BRAHMIN	36	18.0%
JAIN	4	2.0%
JATT	60	30.0%
KAPOOR	2	1.0%
KHATRI	28	14.0%
KUMBI	1	0.5%
MAJBI	17	8.5%
PANDIT	7	3.5%
RAJPUT	4	2.0%
RAMDASIA	5	2.5%
RAMGARHIA	2	1.0%
SUNNI	5	2.5%
Total	200	100.0%

FIG 6. CASTE BASED DISTRIBUTION OF THALASSEMIA



A caste-wise analysis was also done in the patients, and it was concluded that thalassemia is most prevalent amongst Jatts. 60 patients were jatts, 7 were Ad-Dharmi, 12 belonged to Arora cate, 10 were Baniya, 4 were Jain, 2 belonged to Kapoor caste, 28 were Khatri, 1 was Kumbi, 17 belonged Majbi caste, 7 were Pandit, 4 were Rajput, 5 were Ramdasia, 2 were Ramgarhia and 5 belonged to Sunni caste as shown in fig6 and table 1.

FIG 7: FAMILY HISTORY

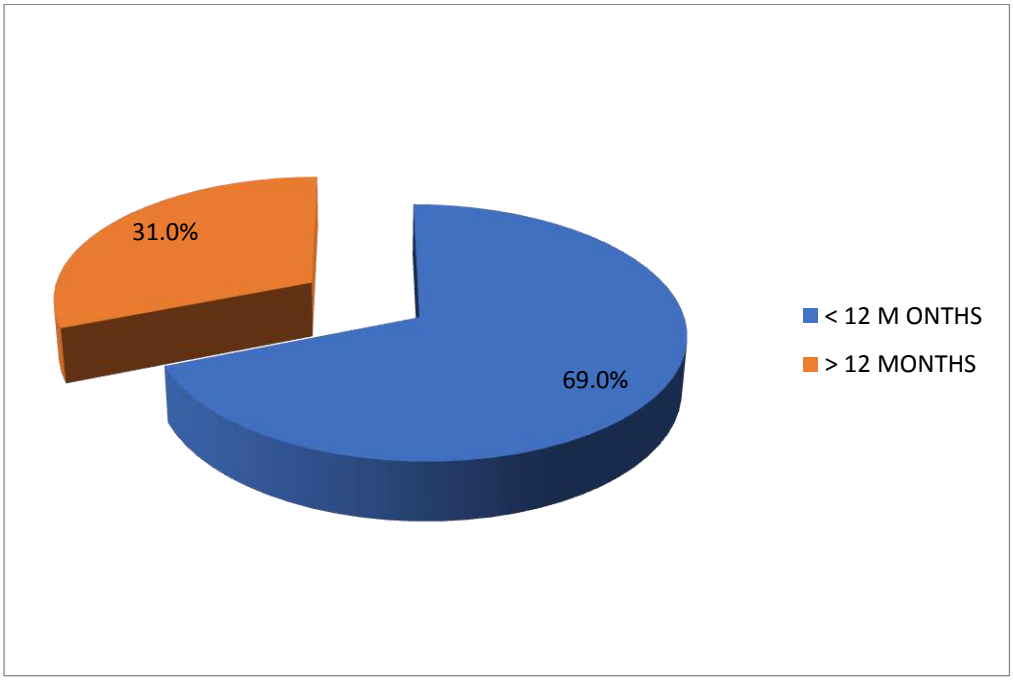


103 patients had family history of thalassemia whereas 97 had no history of thalassemia or atleast they were not aware of the same as shown in FIG 7.

TABLE 2: AGE OF FIRST TRANSFUSION

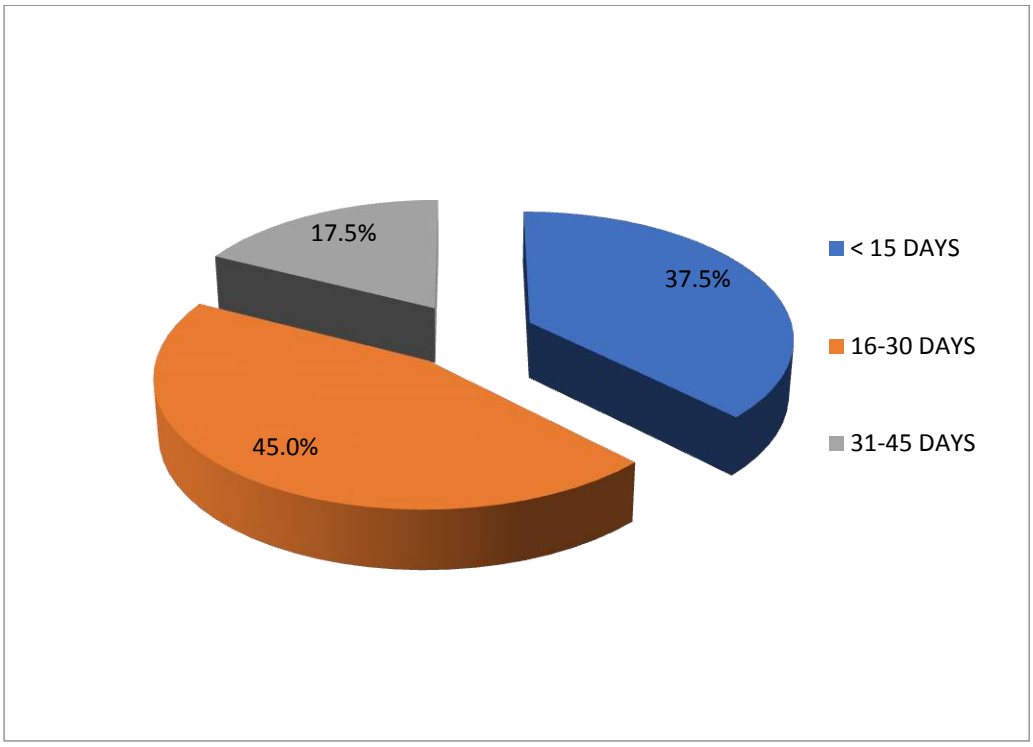
YEARS OF TRANSFUSION	No. OF CASES	PERCENTAGE
< 12 MONTHS	138	69.0%
> 12 MONTHS	62	31.0%

FIG 8: AGE AT THE START OF FIRST TRANSFUSION



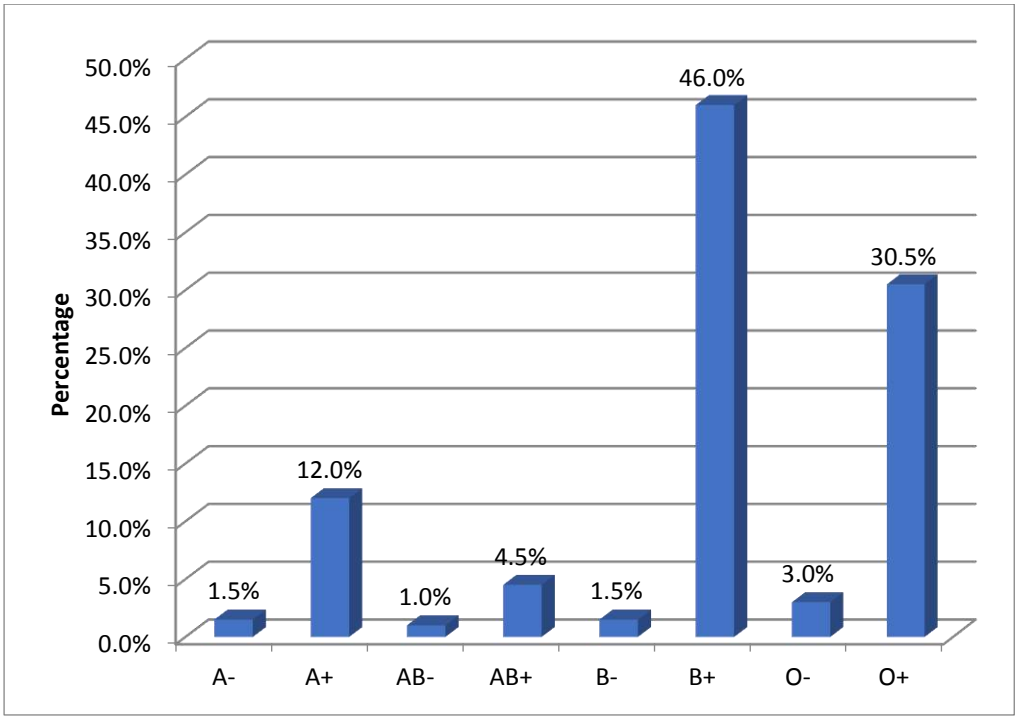
138 patients had their first transfusion at the age of <12 months whereas only 62 patients had their first transfusion at the age of >12months as shown in table 2 and fig8.

FIG 9: TRANSFUSION REQUIREMENT IN PATIENTS



According to transfusion requirement of the patients 3 categories were made. 75 patients required transfusion in less than 15 days, 90 patients required transfusion within span of 16-30 days and 35 patients required transfusion within span of 31-45 days as depicted in fig 9.

FIG 10: DISTRIBUTION OF BLOOD GROUPS.



According to Blood group of the patients among RhD positive A were 24, 61 among them were O, 9 were AB and 92 amongst them were B. On the other hand among RhD negative 3 were A, 3 were B, 6 were O and were AB. The distribution of blood groups is as shown in fig 10.

TABLE 3: DAT RESULTS IN PATIENTS

DAT	NO. OF CASES	PERCENTAGE
NEGATIVE	149	74.5%
POSITIVE	51	25.5%
Total	200	100.0%

DAT panel was put on IgG+C3d cards, 51 had positive results and 149 had negative results as depicted in Fig 11 and table 3.

FIG 11: DAT RESULTS IN PATIENTS

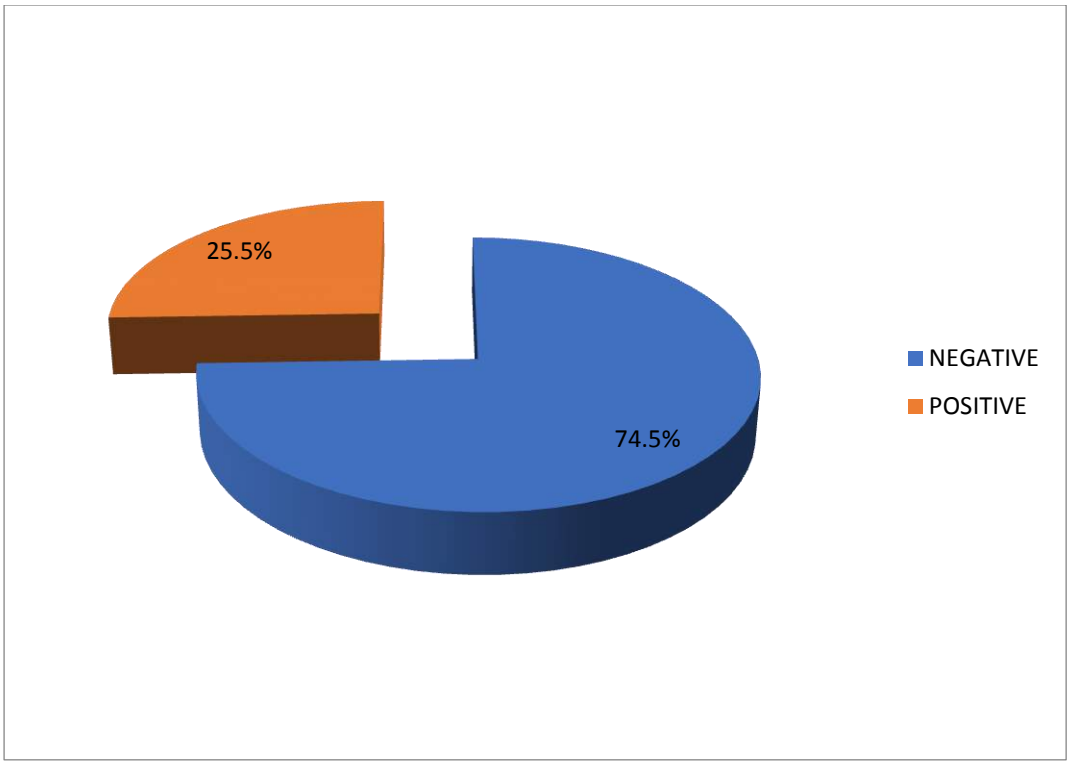
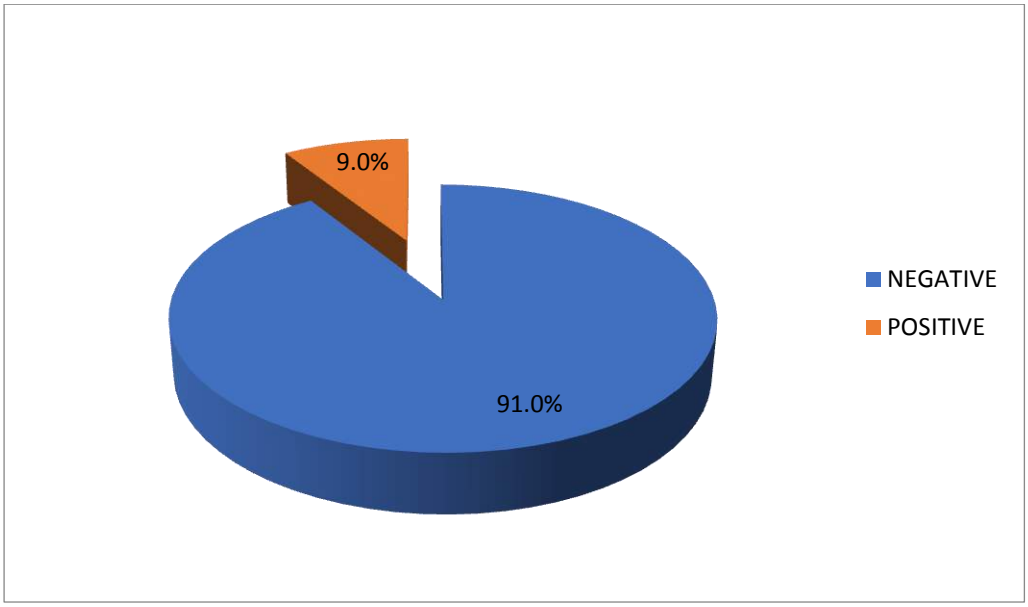


TABLE 4: IAT AND SCREENING RESULTS IN PATIENTS

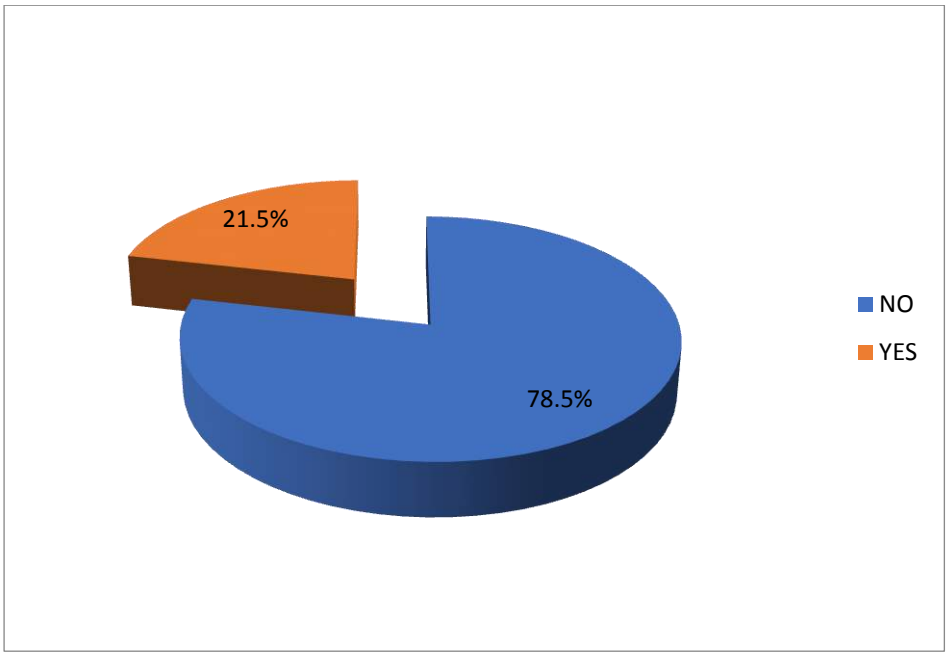
IAT and 3-Cell Panel	NO. OF CASES	PERCENTAGE
NEGATIVE	182	91.0%
POSITIVE	18	9.0%
TOTAL	200	100.0%

FIG. 12: IAT AND ANTIBODY SCREEN PANEL



Amongst 200 patients 18 patients had IAT positive and 182 patients had negative IAT on IgG+C3d BioRad cards. Antibody screen 3-cell panel was put on IAT positive sample using commercially available panel cells as shown in fig.12 and table 4.

FIG 13. NUMBER OF SPLENCECTOMIZED PATIENTS



Out of 200 patients 43 had splenectomy done as shown in fig 13.

TABLE 5- PATIENTS WITH ALLOANTIBODIES

11- CELL	NO. OF CASES	PERCENTAGE
ANTI-C	1	0.5%
ANTI-c,K	1	0.5%
ANTI-E	2	1.0%
ANTI-Jk^a	1	0.5%
ANTI-K	8	4.0%
ANTI-K,E	1	0.5%
ANTI-K. Kp^a	1	0.5%
ANTI-Le^a	1	0.5%
ANTI-Le^b	1	0.5%
ANTI E,C^w	1	0.5%
NEGATIVE	182	91.0%
Total	200	100.0%

Out of 200 patients 18 patients developed alloantibodies and 182 patients had negative results. 4 patients had multiple antibodies whereas 14 patients had single antibody as depicted in table 13 and fig.13. Detail of patients is explained in table 14. A total of 22 alloantibodies were identified 11 were anti-K, 4 were Anti-E and 1 patient of Anti-Jk^a, Anti- Le^a, Anti- Le^b, Anti-C^w, Anti Kp^a, Anti-C and anti-c each as shown in table 6. And distribution alloantibodies is depicted in fig 14.

FIG 14: DISTRIBUTION OF ALLOANTIBODIES AMONGST THALASSEMIA PATIENTS

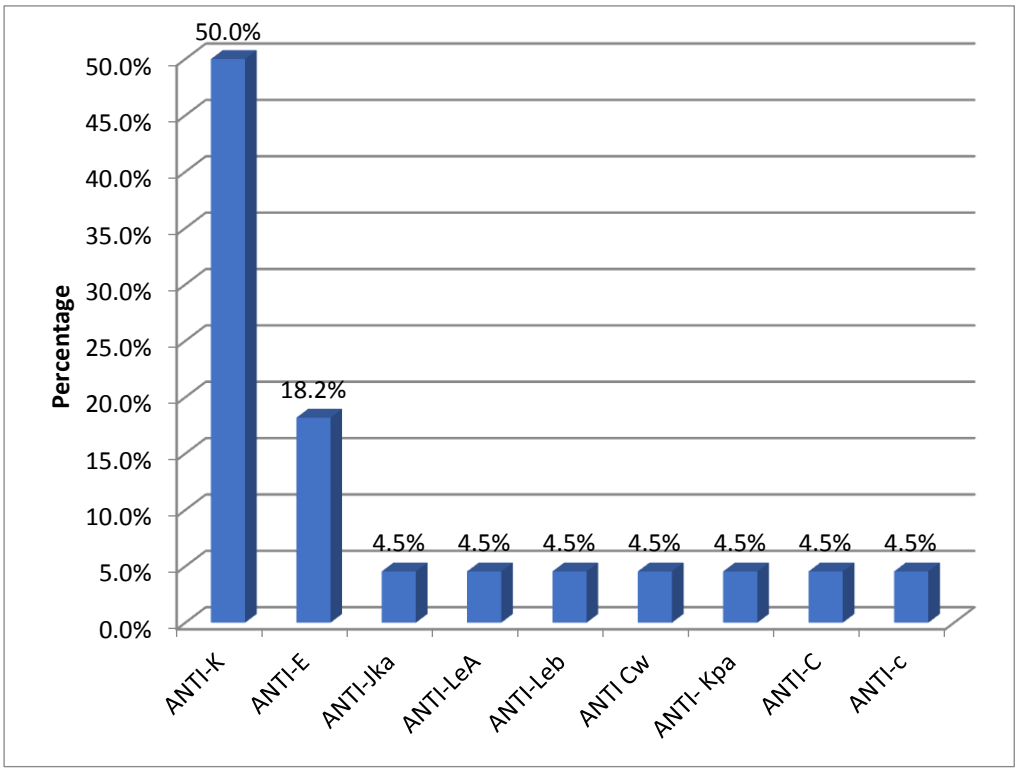


TABLE 6: DETAILS OF ALLOIMMUNIZAD PATIENTS.

S. NO	AGE	1ST TRANSFUSION (IN MONTHS)	TOTAL TRANSFUSION	DAT	SPLENCTOMY	ANTIBODY
Case 1	12	18	144	POSITIVE	NO	ANTI-K
Case 2	17	12	188	NEGATIVE	NO	ANTI-K
Case3	14	12	150	NEGATIVE	NO	ANTI-K
Case 4	12	6	160	NEGATIVE	NO	ANTI-K
Case 5	23	10	389	POSITIVE	YES	ANTI-K,E
Case 6	18	18	180	POSITIVE	NO	ANTI-Jk^b
Case 7	3	7	90	POSITIVE	NO	ANTI-Le^b
Case 8	14	4	166	NEGATIVE	NO	ANTI-K
Case 9	14	8	135	POSITIVE	NO	ANTI-E, C^w
Case 10	15	8	155	POSITIVE	NO	ANTI-E
Case 11	21	10	220	NEGATIVE	YES	ANTI-c,K
Case 12	17	7	170	POSITIVE	NO	ANTI-K
Case 13	15	18	158	NEGATIVE	NO	ANTI-Le^a
Case 14	25	6	330	POSITIVE	YES	ANTI K
Case 15	19	10	203	POSITIVE	NO	ANTI-K,Kp^a
Case 16	7	18	70	POSITIVE	NO	ANTI-C
Case 17	16	16	210	POSITIVE	NO	ANTI-K
Case 18	15	15	190	NEGATIVE	NO	ANTI-E

11 of the alloimmunized patients also had DAT positive and 6 of these patients also had Autoantibodies. 3 of the alloimmunized patients had splenectomy done.

TABLE 7: FREQUENCY OF AUTOIMMUNIZATION IN REALTION TO MEAN AGE, FIRST TRANSFUSION AND TOTAL UNITS TRANFUSED.

AUTOIMMUNIZATION	NEGATIVE		POSITIVE		Z	p-value
	Mean	SD	Mean	SD		
AGE	14.18	8.48	20.96	6.94	-3.953	**0.000**
YEARS OF TRANSFUSION	8.70	3.86	8.48	3.34	0.277	0.782
UNITS TRANSFUSED	189.21	127.11	291.63	115.79	-3.938	**0.000**

Of 200 patients 51 patients developed positive DAT and out of 51 patients 27 developed autoantibodies as determined by positive autocontrol on gel card (IgG+C3d). Mean age of development of autoantibodies was 20.96 years which was significantly higher than patients with no autoimmunization (14.18years) (p=0.000). Number of units transfused was significantly higher in patients with autoimmunization (291.63) than in patients no autoimmunization 189.21 years) (p=0.000) as shown in table 7.

TABLE 8: FREQUENCY OF AUTOIMMUNIZATION IN PATIENTS WITH SPLENECTOMY.

		AUTOIMMUNIZATION				Total	Chi-square value	p-value
		NEGATIVE		POSITIVE				
SPLENECTOMY	NO	146	84.4%	11	40.7%	157	26.369	**0.001**
	YES	27	15.6%	16	59.3%	43		
Total		187	108.1%	27	100.0%	200		

Patients who have undergone splenectomy had higher prevalence of autoantibodies (59.3%) as compared to patients with no autoimmunization (15.6%) (p=0.001) as shown in table 8.

TABLE 9: FREQUENCY OF AUTOIMMINZATION IN RELATION OF AGE OF FIRST TRANSFUSION.

		AUTOIMMINZATION				Total	Chi-square value	p-value
		NEGATIVE		POSITIVE				
YEARS OF TRANSFUSION GROUP	< 12 months	145	83.8%	24	88.9%	169	0.459	0.498
	> 12 months	28	16.2%	3	11.1%	31		
Total		173	100.0%	27	100.0%	200		

As patients were divided into 2 groups. Group 1 included patients who had their first transfusion at the age of <12 months and Group 2 included patients who had first transfusion at the age of >12months. Age of starting of transfusion had no effect on autoimmunization and the p-value was not significant for the same as shown in table 9.

TABLE 10: FREQUENCY OF ALLOIMMUNIZATION IN THALASSEMIA PATIENTS IN RELATION TO MEAN AGE OF PATIENTS, MEAN AGE OF FIRST TRANSFUSION AND TOTAL UNITS TRANFUSED

ALLOIMMUNIZATION	NEGATIVE		POSITIVE		Z	p-value
	Mean	SD	Mean	SD		
AGE	15.07	8.87	15.39	5.20	-0.152	0.879
YEARS OF TRANSFUSION	8.41	3.59	11.28	4.79	-3.127	**0.002**
UNITS TRANSFUSED	204.94	134.41	183.78	74.89	0.657	0.512

Mean years of transfusion was 15.39 years for development of alloantibodies when compared to non-alloimmunized patients 15.07 years which is not significant. Mean age of first transfusion was 11.28 months which was significant when compared to non-alloimmunized patients (8.41 months) (0.002) as depicted in table 10.

TABLE 11: FREQUENCY OF ALLOIMMUNIZATION IN RELATION TO AGE OF FIRST TRANSFUSION.

		ALLOIMMUNIZATION				Total	Chi-square value	p-value
		NEGATIVE		POSITIVE				
YEARS OF TRANSFUSION GROUP	< 12 months	159	87.4%	10	55.6%	169	12.652	**0.001**
	> 12 months	23	12.6%	8	44.4%	31		
Total		182	100.0%	18	100.0%	200		

Prevalence of alloantibodies was significantly lower in patients who received their first transfusion before 12months (p=0.001) as shown in table 11.

TABLE 12: FREQUENCY OF ALLOIMMUNIZATION IN SPLENECTOMIZED PATIENTS.

		ALLOIMMUNIZATION				Total	Chi-square value	p-value
		NEGATIVE		POSITIVE				
SPLENECTOMY	NO	143	78.6%	14	77.8%	157	0.006	0.938
	YES	39	21.4%	4	22.2%	43		
Total		182	100.0%	18	100.0%	200		

However, splenectomy had no effect on alloimmunization as shown in table 12.

DISCUSSION

Alloimmunization is the process of formation on antibodies against the RBC antigens. Process of alloimmunization is usually stimulated by transfusion of blood and blood products or pregnancy. Alloimmunization is one of most common complication faced by Blood Transfusion Services in completing compatibility testing in a timely manner and even obtaining antigen-negative blood units.

The risk of an individual patient developing a non-ABO alloantibody depends upon many factors, including the patient's underlying diseases, the cause of anemia, the cumulative number of transfusions, and the immunogenicity of the non-self RBC antigens to which the patient is exposed.[54] There has been substantial research to identify the clinical and biological factors associated with responders. These may include genetics, the inflammatory state of the patient, sex, and age.[55]

Other than RBC alloimmunization, immunologic complications of repeated RBC transfusions include -Difficulties obtaining compatible blood, development of autoantibodies, acute or delayed hemolytic transfusion reactions and hemolytic disease of the newborn.[56]

In our study the overall rate of alloimmunization was 9%. A low rate of alloimmunization can be expected when there is homogeneity of RBC antigens between donor and recipient. [7]

Previous studies conducted by Singer et al. reported rate of alloimmiunization as high as 22%.[7] There is huge variability of results when it comes to rate of alloimmunization. It ranges from as low as 3.79% to as high as 32.5%. Previous data on presumed homogenous populations in Greece and Italy showed an overall low rate (5% to 10%) of alloimmunization. [6,57,58]

TABLE 13- RATE OF ALLOIMMUNIZATON AND ALLOIMMUNIZATION IN PREVIOUSLY CONDUCTED STUDIES ON THALASSEMIA PATIENTS

AUTHORS	RATE OF AUTOIMMUNIZATION	RATE OF ALLOIMMUNIZATION
Singer et al. (2000) [7]	25%	22.%
Khalid et al(2004) [46]	-	22.7%
Sadeghian et al.(2009) [47]	-	2.87%
Gupta et al(2011) [48]	-	9.98%
Pahuja et al(2013) [49]	0.47%	3.79%
Dhawan et al. (2014) [50]	28.32%	5.64%
Datta et al.(2015) [3]	1%	5.71%
Amal et al.(2020) [52]	16.5%	18%
Waldi et al (2020) [53]	27.5%	32.5%
our study(2021)	13.5%	9%

In our study it was observed that prevalence of thalassemia was higher in urban population (52.5%) as compared to rural population (47.5%) and 95% of patients admitted in our centre belonged to Punjab state.

A caste analysis was done it was observed that thalassemia major was more prevalent amongst Jatts (30%) followed by Brahmin(18%), Khatri (14%), majbi (8.5%), Arora (6%), Baniya(5%), Pandit (3.5%), Ad-dharmi (3.5%), Ramdasia(2.5%), Sunni (2.5%), Rajput(2%), Jain(2%), Kapoor (1%), Ramgarhia(1%) . Least number of case were from kumbi Caste (0.5%).

18 out of 200 patients developed alloantibodies. Most prevalent was Anti-K (50%), followed by Anti-E (4%), Anti Jk^a(4.5), Anti-Le^a (4.5%), Anti- Le^b (4.5%), Anti- C^w (4.5%), Anti-Kp^a(4.5%), Anti-c (4.5%) and Anti-C (4.5%). Out of 22 antibodies 31% antibodies belonged to Rh

Blood group system and 54.5% antibodies were from Kell Blood group system. These findings were in accordance with the previous studies which state that anti-Kell , anti-E and anti-D are the most common in these patients [4,7,47, 59, 60,61,62]. However, we could not find any anti-D antibody. Prevalence of antibodies in different studies is shown in table 13

TABLE 14- TABLE SHOWING PREVALENCE OF ALLOANTIBODIES AMONGST ALLOIMMUNIZED PATIENTS

PREVALENCE OF ANTIBODIES (%)			
ANTIBODY	**PRESENT STUDY**	**DHAWAN et al.**	**DATTA et al.**
ANTI-K	50%	35%	-
ANTI-E	18.2%	17%	21.42%
ANTIJkb	4.5%	19%	7.1
Anti-Le^a	4.5%	-	-
Anti Le^b	4.5%	-	-
Anti-Cw	4.5%	9%	-
ANTI-Kpa	4.5%	-	-
ANTI-c	4.5%	-	-
ANTI-C	4.5%	13%	3.57%
ANTI-D	-	13%	3.57%
ANTI-Xg	-	4%	-
ANTI-Jka	-	-	3.57%
ANTI-s	-	-	3.57%

Mean age of developing autoantibodies was 18.22 years which is significantly higher than patients with no alloantibodies. Age at first transfusion was higher in in alloimmunized (11.28 months) than non-immunized patients (8.41 months) (p=0.002). Transfusion at an early age may offer some immune tolerance and alloimmunization against thalassemia patients. [63,64]

We did not find any co-relation between gender and rate of alloimmunization. Few studies in literature have shown that gender was not a significant factor in the development of alloantibodies.[65,66]

However, some studies conducted by Reisner et al. [67] reported alloimmunization more in females whereas in another study conducted by Saied et al [61] found more alloimmunization in male patients.

The relationship between alloimmunization and total number of units transfused in unknown in thalassemia [68]. No. of units transfused had no significance on alloimmunization but total number of units were significantly higher in patients with autoantibodies which co-relates with the study conducted by Dhawan et al. [50] which also showed more autoimmunization in patients who received more transfusions in their lifetime. However, some studies in literature have reported that patients who received more transfusions are more likely to develop alloantibodies.[69,70]

In present study, 27(13.5%) patients developed autoantibodies. It is a known fact that autoantibodies are formed are more in patients who have undergone splenectomy.[71] Clinical significance of autoantibodies in multiple transfused is debatable but some reports in literature state that warm antibodies can cause clinically significant hemolysis.[72] But others did not find any association. [73] In our study out of 27 patients with autoantibodies, 16 (59.2%) patients had splenectomy done. The possible explanation for this is that lymphocytosis associated with blood transfusion is also associated with elevated levels of serum immunoglobulins, immune complexes and cells expressing surface immunoglobulins,[74,75] suggesting the possible role of absence of spleen and autoantibody formation.

SUMMARY & CONCLUSION

- Rate of alloimmunization in our study was 9% and 85.5% of these antibodies belonged to Rh and Kell blood group systems.
- Rh and kell blood group system antibodies are known for causing severe HTRs. So patient who need multiple transfusion as in our case it is thalassemia patients, RBC antigen typing before first transfusion is a must.
- Early diagnosis and early institution of transfusion therapy may also prevent alloimmunization.
- Leucodepleted Antigen matched blood (at least for Rh and kell) should be given to these patients to avoid alloimmunization and HTR.
- Regular antibody- screening should be done in these patients to check for new formation of new antibodies.
- When considering splenectomy in such patients, a potential higher risk for RBC should be taken in account.
- Although very few studies reported hemolysis associated with formation of autoantibodies, these patients should undergo regular serological investigations to prevent any unforeseen complications.
- Blood Centres should recruit regular donors to avail compatible blood units for patients.

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S. No	AGE	SEX	ABO&Rh	MARRITAL STATUS	LOCALITY	REGION	RELIGION	CASTE	FAMILY HISTORY	YEARS OF TRANSFUSION	UNITS TRANSFUSED	TRANSFUSION REQUIREMENT	DAT	IAT	3- CELL	11- CELL	AUTOCONTROL	SPLENECTOMY
1	12Yr	F	O+	UNMARRIED	URBAN	PUNJAB	HINDU	ARORA	NONE	SINCE AGE OF 18 MONTHS	144	18-25 DAYS	POSITIVE	POSITIVE	POSITIVE	ANTI-K	NEGATIVE	NO
2	2	F	B +	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	PRESENT	7 MONTHS	18	35-40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
3	38	F	B+	MARRIED	RURAL	PUNJAB	SIKH	ARORA	PRESENT	18 MONTHS	576	15 DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
4	17	M	O+	UNMARRIED	RURAL	PUNJAB	SIKH	KHATRI	PRESENT	3 MONTHS	180	15 DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
5	17Yr	M	O+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	12MONTHS	188	15DAYS	NEGATIVE	POSITIVE	POSITIVE	ANTI-K	NEGATIVE	NO
6	10MONTHS	M	AB+	UNMARRIED	RURAL	BIHAR	HINDU	KUMBI	NONE	2MONTHS	4	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
7	17	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	6 MONTHS	190	15 DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
8	15	M	O+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	NONE	4 MONTHS	240	15 DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
9	9	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	KHATRI	NONE	8MONTHS	216	15 DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
10	23	F	O-	UNMARRIED	RURAL	PUNJAB	HINDU	BANIYA	PRESENT	24MONTHS	431	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
11	13	F	O+	UNMARRIED	URBAN	PUNJAB	SIKH	KHATRI	NONE	6MONTHS	156	20-21 DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
12	4	F	B+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	NONE	24MONTHS	66	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
13	10	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	12MONTHS	160	20-21	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
14	1	M	A+	UNMARRIED	URBAN	DELHI	HINDU	BRAHMIN	NONE`	6 MONTHS	9	30-40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
15	6	M	O+	UNMARRIED	URBAN	PUNJAB	MUSLIM	SUNNI	PRESENT	12MONTHS	50	30-40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
16	14YRS	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	PANDIT	PRESENT	12MONTHS	150	20DAYS	NEGATIVE	POSITIVE	POSITIVE	ANTI-K	NEGATIVE	NO
17	11	M	A+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	PRESENT	12MONTHS	130	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
18	10	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	BRAHMIN	NONE	6 ,MONTHS	120	20-25 DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
19	11	F	O+	UNMARRIED	URBAN	PUNJAB	HINDU	JATT	PRESENT	12MONTHS	160	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
20	15	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	8MONTHS	200	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
21	5	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	RAJPUT	PRESENT	7MONTHS	150	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
22	12YRS	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	KHATRI	PRESENT	6MONTHS	160	20 DAY	NEGATIVE	POSITIVE	POSITIVE	ANTI-K	NEGATIVE	NO
23	26	M	AB+	MARRIED	RURAL	PUNJAB	HINDU	BRAHMIN	PRESENT	8MONTHS	426	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
24	27	M	B+	UNMARRIED	URBAN	PUNJAB	SIKH	KHATRI	NONE	6MONTHGS	468	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
25	13	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	BANIYA	PRESENT	8MONTHS	190	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
26	14	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NONE	6 MONTHS	200	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
27	9	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	BANIYA	PRESENT	12MONTHS	110	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
28	6	M	A+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	NONE	24MONTHS	61	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
29	10	F	B+	UNMARRIED	URBAN	PUNJAB	HINDU	KAPOOR	PRESENT	5MONTHS	110	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
30	10	F	B+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	PRESENT	8MOMNTS	135	20-250DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
31	10YRS	F	B+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NONE	10MONTHS	140	20-25	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
32	16YRS	F	O+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	NONE	3 MONTHS	210	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
33	16YRS	F	B+	UNMARRIED	URBAN	PUNJAB	HINDU	KAPOOR	NONE	6MONTHS	135	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
34	18YRS	F	A+	UNMARRIED	RURAL	PUNJAB	HINDU	ARORA	NONE	12MONTHS	175	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
35	17YRS	F	B+	UNMARRIED	URBAN	PUNJAB	SIKH	ARORA	PRESENT	4MONTHS	160	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
36	25YRS	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	BRAHMIN	PRESENT	8MONTHS	386	15 DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
37	22yrs	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	PRESENT	12MONTHS	400	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
38	4YRS	F	0-	UNMARRIED	URBAN	PUNJAB	HINDU	KHATRI	PRESENT	6MONTHS	125	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
39	5YRS	M	A-	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NONE	8MOMTHS	65	25DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	NO
40	27YRS	M	B+	UNMARRIED	URBAN	HIMACHAL PRADESH	HINDU	BRAHMIN	NONE	7MOMNTHS	513	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
41	16YRS	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	BANIYA	NONE	6MOMNTHS	146	20 DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
42	20	F	A+	UNMARRIED	RURAL	PUNJAB	HINDU	MAJBI	NONE	8MONTHS	168	22DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
43	8YRS	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	10MONTS	168	30DYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
44	22YRS	M	B+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	PRESENT	6MONTHS	482	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
45	10YRS	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	MAJBI	NONE	12MONTHS	175	28DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
46	14YRS	M	A+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	PRESENT	6MONTHS	135	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
47	21YRS	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NONR	8MONTHS	350	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
48	27YRS	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NONE	10MONTHS	436	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
49	24YRS	F	O+	UNMARRIED	URBAN	HARYANA	HINDU	KHATRI	NINE	6MONTHS	430	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
50	19YRS	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NONE	12MONTHS	180	25DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
51	25YRS	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	PANDIT	PRESENT	6MONTHS	412	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
52	23YRS	M	B-	UNMARRIED	URBAN	PUNJAB	HINDU	ARORA	PRESENT	10MONTHS	389	15DAYS	POSITIVE	POSITIVE	POSITIVE	ANTI-K,E	POSITIVE	YES
53	19	F	O-	UNMARRIED	URBAN	PUNJAB	HINDU	AD-DHARMI	NONE	15MONTHS	186	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
54	17	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	RAMGARHIA	PRESENT	7MONTHS	165	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
55	15	M	B+	UNMARRIED	URBAN	PUNJAB	SIKH	KHATRI	PRESENT	6MOMTHS	149	20DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
56	5YRS	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	AD-DHARMI	NONE	8MONTHS	105	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
57	23YRS	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	RAMDASIA	NONE	10MONTHS	359	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
58	3YRS	F	B+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	PRESENT	9MONTHS	101	35DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
59	28	M	O+	MARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NONE	6MONTHS	483	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES

S. No	AGE	SEX	ABO&Rh	MARRITAL STATUS	LOCALITY	REGION	RELIGION	CASTE	FAMILY HISTORY	YEARS OF TRANSFUSION	UNITS TRANSFUSED	TRANSFUSION REQUIREMENT	DAT	IAT	3- CELL	11- CELL	AUTOCONTROL	SPLENECTOMY
60	28	M	B+	MARRIED	RURAL	PUNJAB	JAIN	JAIN	NONE	8MONTHS	489	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
61	14	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	MAJBI	NONE	15MONTHS	160	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
62	19	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NONE	8MONTHS	200	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
63	3	M	O+	UNMARRIED	RURAL	PUNJAB	HINDU	BANIYA	PRESENT	10MONTHS	90	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
64	21	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	MAJBI	PRESENT	6MONTHS	360	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	NO
65	15	M	A+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	NO	10MONTHS	170	25DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
66	2	M	A+	UNMARRIED	URBAN	PUNJAB	MUSLIM	SUNNI	PRESENT	6MONTHS	90	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
67	18	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	MAJBI	NONE	8MONTHS	160	25DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
68	20	M	A+	UNMARRIED	URBAN	PUNJAB	HINDU	KHATRI	PRESENT	10MOMTHS	302	20DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	NO
69	28	M	O+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	NONE	6MONTHS	426	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
70	10	F	O+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NONE	9MONTHS	180	20DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
71	19	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	PANDIT	PRESENT	6MOMTHS	190	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
72	8	M	B-	UNMARRIED	URBAN	PUNJAB	SIKH	KHATRI	PRESENT	8MONTHS	156	30DSAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
73	23	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	MAJBI	PRESENT	10MONTHS	350	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
74	3	M	A+	UNMARRIED	URBAN	PUNJAB	MUSLIM	SUNNI	PRESENT	6MONTHS	85	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
75	29	F	B+	MARRIED	URBAN	PUNJAB	SIKH	JATT	NONE	8MONTHS	389	20DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
76	23	M	A+	UNMARRIED	RURAL	PUNJAB	HINDU	MAJBI	NONE	6MOMNTHS	400	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
77	9	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	BANIYA	NONE	10MONTHS	180	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
78	17	F	AB+	UNMARRIED	RURAL	PUNJAB	HINDU	AD-DHARMI	PRESENT	6MOMNTHS	150	20DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
79	34	F	O+	MARRIED	URBAN	PUNJAB	SIKH	JATT	present	8months	450	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
80	23	F	O-	UNMARRIED	RURAL	PUNJAB	HINDU	BANIYA	PRESENT	6MONTHS	355	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
81	18	F	A+	UNMARRIED	RURAL	PUNJAB	SIKH	RAMDASIA	PRESENT	18MONTHS	180	20DAYS	POSITIVE	POSITIVE	POSITIVE	ANTI-Jka	POSITIVE	NO
82	17	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	NONE	10MONTHS	150	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
83	20	F	A+	UNMARRIED	RURAL	PUNJAB	HINDU	RAMDASIA	NONE	8MONTHS	286	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
84	3	F	AB+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	NONE	7MONTHS	90	40DAYS	POSITIVE	POSITIVE	POSITIVE	ANTI-Leb	POSITIVE	NO
85	23	M	A+	UNMARRIED	RURAL	PUNJAB	HINDU	MAJBI	PRESENT	10MONTHS	349	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
86	3	F	B+	UNMARRIED	URBAN	PUNJAB	HINDU	MAJBI	NONE	8MOMTHS	90	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
87	11	F	AB+	UNMARRIED	URBAN	PUNJAB	HINDU	PANDIT	PRESENT	6MONTHS	110	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
88	20	F	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	NONE	10MONTHS	298	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
89	6	M	O+	UNMARRIED	RURAL	PUNJAB	MUSLIM	SUNNI	PRESENT	6MONTHS	159	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
90	7	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	KHATRI	NONE	5MONTHS	180	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
91	1	F	O+	UNMARRIED	URBAN	PUNJAB	JAIN	JAIN	NONE	7MOMTHS	15	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
92	12	M	O+	UNMARRIED	RURAL	PUNJAB	HINDU	RAJPUT	PRESENT	4MONTHS	126	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
93	1	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	RAJPUT	PRESENT	6MONTHS	7	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
94	25	F	O+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	NONE	6MONTHS	410	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
95	16	F	B+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NO	9MONTHS	170	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
96	14	F	B+	UNMARRIED	URBAN	PUNJAB	SIKH	KHATRI	NO	10MONTHS	145	20DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	NO
97	17	F	B+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	PRESENT	5MONTHS	168	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	NO
98	17	F	B+	UNMARRIED	RURAL	PUNJAB	SIKH	KHATRI	NONE	6MONTHS	155	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
99	16	F	O+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	4MONTHS	180	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	NO
100	23	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	PANDIT	NONE	7MONTHS	348	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
101	13	F	O+	UNMARRIED	URBAN	PUNJAB	SIKH	KHATRI	NONE	8MONTHS	120	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
102	40	M	A+	MARRIED	URBAN	PUNJAB	SIKH	JATT	PRESENT	6MONTHS	512	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
103	17	M	A+	UNMARRIED	URBAN	PUNJAB	JAIN	JAIN	NONE	7MONTHS	165	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
104	10	F	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	6MONTHS	110	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
105	20	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	ARORA	PRESENT	7MONTHS	270	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
106	20	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	MAJBI	PRESENT	6MONTHS	288	20DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
107	14	F	B+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	NONE	4MONTHS	166	20DAYS	NEGATIVE	POSITIVE	POSITIVE	ANTI-K	NEGATIVE	NO
108	18	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	NONE	5MONTHS	180	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
109	38	F	B+	MARRIED	RURAL	PUNJAB	SIKH	JATT	NONE	6MONTHS	482	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
110	11	M	O+	UNMARRIED	RURAL	PUNJAB	SIKH	ARORA	PRESENT	7MONTHS	110	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
111	16	F	B+	UNMARRIED	RURAL	PUNJAB	HINDU	MAJBI	NONE	8MONTHS	180	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
112	7	M	A+	UNMARRIED	RURAL	PUNJAB	SIKH	MAJBI	PRESENT	6MOMTHS	150	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
113	16	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	BANIYA	PRESENT	6MOMTHS	165	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
114	7	M	O+	UNMARRIED	RURAL	PUNJAB	HINDU	MAJBI	NONE	7MONTHS	165	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
115	31	M	O+	MARRIED	URBAN	PUNJAB	SIKH	JATT	PRESENT	4MONTHS	489	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
116	21	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	RAMDASIA	NONE	6MONTHS	260	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES

S. No	AGE	SEX	ABO&Rh	MARRITAL STATUS	LOCALITY	REGION	RELIGION	CASTE	FAMILY HISTORY	YEARS OF TRANSFUSION	UNITS TRANSFUSED	TRANSFUSION REQUIREMENT	DAT	IAT	3- CELL	11- CELL	AUTOCONTROL	SPLENECTOMY
117	3	F	B+	UNMARRIED	URBAN	PUNJAB	HINDU	RAMDASIA	PRESENT	7MONTHS	175	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
118	9	M	AB+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	5MONTHS	150	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
119	35	F	B+	MARRIED	URBAN	PUNJAB	SIKH	JATT	PRESENT	8MONTHS	459	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
120	12	F	O+	UNMARRIED	URBAN	PUNJAB	HNDU	ARORA	NONE	18MONTHS	144	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
121	35	F	B+	MARRIED	URBAN	PUNJAB	SIKH	AD-DHARMI	NONE	6MOMTHS	468	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
122	15	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	5MONTHS	150	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
123	22	M	AB+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NONE	6MONTHS	290	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
124	19	F	O+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	NONE	6MONTHS	180	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
125	21	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	BRAHMIN	PRESENT	8MONTHS	280	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
126	4	F	O-	UNMARRIED	URBAN	PUNJAB	HINDU	KHATRI	NONE	9MONTHS	130	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
127	14	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	KHATRI	PRESENT	6MOMNTHS	160	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
128	10	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	NONE	6MONTHS	140	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
129	18	F	A+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	6MONTHS	175	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	NO
130	24	F	AB+	UNMARRIED	URBAN	PUNJAB	HINDU	KHATRI	PRESENT	7MONTHS	310	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
131	26	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	KHATRI	NONE	5MONTHS	350	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
132	5	M	O+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	NONE	5MONTHS	130	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
133	8	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	KHATRI	PRESENT	8MONTHS	145	25days	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
134	12	F	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	18MONTHS	110	35DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
135	14	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	PRESENT	8MONTHS	135	20DAYS	POSITIVE	POSITIVE	POSITIVE	ANTIE,Cw	POSITIVE	NO
136	15	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	BANIYA	NONE	8MONTHS	155	20DAYS	POSITIVE	POSITIVE	POSITIVE	ANTI-E	NEGATIVE	NO
137	21	M	O+	UNMARRIED	RURAL	PUNJAB	MUSLIM	SUNNI	PRESSENT	10MONTHS	220	15DAYS	NEGATIVE	POSITIVE	POSITIVE	ANTI-c,K	NEGATIVE	YES
138	17	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	7MONTHS	170	15DAYS	POSITIVE	POSITIVE	POSITIVE	ANTI-K	NEGATIVE	NO
139	15	F	A+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	NONE	18MONTHS	158	20DAYS	NEGATIVE	POSITIVE	POSITIVE	ANTI-LeA	NEGATIVE	NO
140	25	M	AB+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	PRESENT	6MONTHS	330	15DAYS	POSITIVE	POSITIVE	POSITIVE	ANTI-K	POSITIVE	YES
141	2	F	O+	UNMARRIED	URBAN	PUNJAB	HINDU	JAIN	PRESENT	18MONTHS	23	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
142	5	F	O+	UNMARRIED	RURAL	PUNJAB	SIKH	ARORA	NONE	8MONTHS	55	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
143	5	F	O+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	PRESENT	10MONTHS	40	40 DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
144	24	M	O+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	7MONTHS	320	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
145	7	F	O+	UNMARRIED	URBAN	UTTARAKHAND	HINDU	ARORA	NONE	1YEAR	156	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
146	27	M	B+	UNMARRIED	URBAN	PUNJAB	SIKH	KHATRI	NONE	8MONTHS	366	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
147	30	M	B+	MARRIED	URBAN	PUNJAB	SIKH	JATT	PRESENT	9MONTHS	465	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
148	5	M	O+	UNMARRIED	RURAL	PUNJAB	SIKH	ARORA	NONE	10MONTHS	59	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
149	1	M	O+	UNMARRIED	RURAL	UTTAR PRADESH	HINDU	PANDIT	PRESENT	6MONTHS	10	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
150	12	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	KHATRI	PRESENT	9MONTHS	150	35 DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
151	4	F	B+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NONE	8MONTHS	126	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
152	9	F	B+	UNMARRIED	RURAL	PUNJAB	HINDU	BRAHMIN	NONE	10MONTHS	156	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
153	19	M	O+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	10MONTHS	203	15DAYS	POSITIVE	POSITIVE	POSITIVE	ANTI-K. Kpa	NEGATIVE	NO
154	23	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	PRESENT	9MONTHS	296	20DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
155	4	F	A+	UNMARRIED	RURAL	PUNJAB	HINDU	AD-DHARMI	NONE	18MONTHS	56	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
156	37	F	B+	UNMARRIED	RURAL	PUNJAB	HINDU	AD-DHARMI	PRESENT	7MONTHS	426	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
157	7	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	RAJPUT	NONE	10MONTHS	103	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
158	3	M	O-	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	18MONTHS	30	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
159	10	M	AB-	UNMARRIED	RURAL	PUNJAB	SIKH	RAMGARHIA	PRESENT	8MONTHS	90	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
160	18	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	KHATRI	NONE	9MONTHS	190	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
161	15	M	O+	UNMARRIED	RURAL	PUNJAB	HINDU	BANIYA	NONE	10MONTHS	156	30DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
162	18	M	O+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	9MONTHS	210	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
163	1	F	O+	UNMARRIED	RURAL	PUNJAB	HINDU	BRAHMIN	NONE	8MONTHS	5	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
164	33	M	O+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	PRESENT	10MONTHS	436	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
165	18	F	O+	UNMARRIED	RURAL	JAMMU AND KASHMIR	HINDU	BRAHMIN	NONE	8MONTHS	200	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
166	12	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	18MONTHS	150	25DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
167	10	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	NONE	9MONTHS	130	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
168	5	F	B+	UNMARRIED	URBAN	PUNJAB	HINDU	AD-DHARMI	NONE	6MONTHS	60	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
169	17	F	B+	UNMARRIED	RURAL	PUNJAB	HINDU	PANDIT	PRESENT	10MONTHS	119	30DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
170	17	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	KHATRI	NONE	6MOMNTHS	180	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
171	28	F	B+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	PRESENT	9MONTHS	392	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
172	10	F	B+	UNMARRIED	RURAL	PUNJAB	HINDU	MAJBI	PRESENT	10MONTHS	95	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
173	9	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	KHATRI	PRESENT	9MONTHS	103	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO

S. No	AGE	SEX	ABO&Rh	MARRITAL STATUS	LOCALITY	REGION	RELIGION	CASTE	FAMILY HISTORY	YEARS OF TRANSFUSION	UNITS TRANSFUSED	TRANSFUSION REQUIREMENT	DAT	IAT	3- CELL	11- CELL	AUTOCONTROL	SPLENECTOMY
174	16	F	A+	UNMARRIED	URBAN	PUNJAB	SIKH	KHATRI	NONE	10MONTHS	140	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
175	16	M	A+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	NONE	10MONTHS	167	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
176	7	F	B-	UNMARRIED	RURAL	PUNJAB	SIKH	MAJBI	PRESENT	18MONTHS	70	30DAYS	POSITIVE	POSITIVE	POSITIVE	ANTI-C	NEGATIVE	NO
177	3	M	O+	UNMARRIED	RURAL	BIHAR	HINDU	BRAHMIN	NONE	8MONTHS	66	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
178	14	M	O+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	PRESENT	6MONTHS	105	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
179	2	F	O+	UNMARRIED	URBAN	PUNJAB	HINDU	MAJBI	PRESENT	7MONTHS	25	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
180	6	M	O+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	PRESENT	8MONTHS	85	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
181	21	M	O+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	NONE	10MONTHS	250	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
182	2	M	O+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	NONE	6MONTHS	30	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
183	29	M	B+	UNMARRIED	RURAL	HIMACHAL	HINDU	BRAHMIN	NONE	15MONTHS	398	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	YES
184	17	M	B+	UNMARRIED	URBAN	PUNJAB	HINDU	ARORA	PRESENT	10MONTHS	170	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
185	18	F	B+	UNMARRIED	RURAL	HIMACHAL	HINDU	BRAHMIN	NONE	8MONTHS	190	20FDAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
186	12	M	B+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	NONE	6MONTHS	146	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
187	12	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	10MONTHS	150	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
188	12	F	B+	UNMARRIED	URBAN	PUNJAB	HINDU	BRAHMIN	NONE	10MONTHS	160	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
189	20	F	B+	UNMARRIED	RURAL	PUNJAB	HINDU	MAJBI	PRESENT	9MONTHS	265	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
190	3	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	BRAHMIN	NONE	6MONTHS	50	45DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
191	16	F	B+	UNMARRIED	URBAN	PUNJAB	SIKH	KHATRI	PRESENT	16MONTHS	210	15DAYS	POSITIVE	POSITIVE	POSITIVE	ANTI-K	POSITIVE	NO
192	19	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	10MONTHS	170	25DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
193	5	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	6MONTHS	60	35DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
194	5	M	A+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	NONE	8MOMTHS	59	40DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
195	27	M	A+	UNMARRIED	URBAN	PUNJAB	SIKH	JATT	NONE	10MONTHS	398	15DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	YES
196	15	M	A-	UNMARRIED	URBAN	PUNJAB	HINDU	KHATRI	NONE	15MONTHS	190	15DAYS	NEGATIVE	POSITIVE	POSITIVE	ANTI-E	NEGATIVE	NO
197	12	M	B+	UNMARRIED	RURAL	PUNJAB	SIKH	JATT	PRESENT	6MONTHS	154	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
198	15	M	AB-	UNMARRIED	RURAL	PUNJAB	HINDU	JATT	NONE	8MONTHS	169	30DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
199	20	M	A-	UNMARRIED	RURAL	PUNJAB	HINDU	BRAHMIN	PRESENT	6MOMNTHS	150	20DAYS	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO
200	19	M	B+	UNMARRIED	RURAL	PUNJAB	HINDU	KHATRI	PRESENT	10MONTHS	196	15DAYS	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NO

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Date 20/4/2021

The Principal,
DMC Ludhiana

Subject: **Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Sciences in its meeting held on 22.01.2021 has approved the plan of thesis of **Dr. Manpreet Kataria**, a student of your college for **MD Immuno Haematology & Blood Transfusion** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis

To study alloimmunization and autoimmunization in transfusion dependent thalassemia major patients

Supervisor
Dr. Rajesh Kumar
Prof.,
Dept. of I.H.B.T.,
DMCH, Ludhiana.

Co-Supervisor
Dr. Amarjit Kaur
Prof. & Head,
Dept. of I.H.B.T.,
DMCH, Ludhiana.

Dr. Sonia Gupta
Assoc. Prof.,
Dept. of I.H.B.T.,

The student has to submit the three copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic,(if any).

Dr. S P Singh MS(Oph)
Controller of Examinations

Note :

1. ***If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.***
2. *The student may submit the thesis as per the schedule already conveyed i.e for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examinations 31st May of the year of examination.*

TO STUDY ALLOIMMUNIZATION AND AUTOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA MAJOR PATIENTS

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED IN

PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MD

In

IMMUNOHEMATOLOGY AND BLOOD TRANSFUSION

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES

FARIDKOT

NOVEMBER, 2019

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DEPARTMENT OF IMMUNOHEMATOLOGY AND BLOOD TRANSFUSION
DAYANAND MEDICAL COLLEGE AND HOSPITAL
LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	TO STUDY ALLOIMMUNIZATION AND AUTOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA MAJOR PATIENTS
For the degree of	MD (IMMUNOHEMATOLOGY AND BLOOD TRANSFUSION)
Name of the candidate	Dr. Manpreet Kataria
Supervisor	Dr. Rajesh Kumar, Professor, Department of IHBT, DMCH, Ludhiana
Co-supervisor	Dr. Amarjit Kaur, Professor & Head, Department of IHBT, DMCH, Ludhiana Dr. Sonia Gupta, Associate Professor, Department of IHBT, DMCH, Ludhiana
Institution	Dayanand Medical College and Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

Introduction: Beta thalassemia is characterized by major or total suppression of beta chain synthesis. Lifelong transfusion every 2-4 weeks to maintain hemoglobin levels is required. The development of RBC antibodies both alloantibodies and autoantibodies can significantly complicate the transfusion therapy which further can cause hemolytic type of transfusion reaction.

Aims & Objectives: The primary aim of the present study is to determine the frequency of alloimmunization and autoimmunization among thalassemia major patients.

Material & Methods: This prospective study will be carried out on all the thalassemia major patients registered with the hospital. Clinical and transfusion records of all the patients will be examined for age of patients, age at initiation of transfusion therapy, total number of blood units transfused, transfusion interval, status of splenectomy, or other interventions. Alloantibody screening and identification will be done using 3-cell(Immucor and Biorad). If 3 cell panel positive 11 cell-panel(Bio-rad, Switzerland). To detect autoantibodies, auto-control will be performed by DCT and Auto-Control using polyspecific(IgG+c3d) gel cards.

Clinical Significance: Development of red cell alloantibodies in multi transfused thalassemia patients is one complication in the treatment which can cause hemolytic type of transfusion reaction and causes incompatible cross-matching.

Keywords: Alloimmunization, Autoimmunization, beta thalassemia major, transfusion.

(Signature) Candidate | (Signature) Co-supervisor | (Signature) Co-supervisor | (Signature) Supervisor | (Signature) Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"TO STUDY ALLOIMMUNIZATION AND AUTOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA MAJOR PATIENTS"** exist in the Department of IHBT, DMC&H, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis is genuine and is collected by the candidate herself under our supervision and guidance.

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APPROVAL PROFORMA
BY
RESEARCH & ETHICAL COMMITTEE
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Topic of Thesis	TO STUDY ALLOIMMUNIZATION AND AUTOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA MAJOR PATIENTS
Likely date of appearing in PG examination	May 2022
Date of enrollment	9th May, 2019
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Approved: Yes / ~~No~~

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Chairperson
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Approved: Yes / ~~No~~

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PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

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3	COURSE OF STUDY AND SUBJECT	MD IMMUNOHEMATOLOGY AND BLOOD TRANSFUSION
4	DATE OF ADMISSION OF COURSE	09-05-2019
5	TITLE OF THE TOPIC	TO STUDY ALLOIMMUNIZATION AND AUTOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA MAJOR PATIENTS

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

Beta-thalassemia is a congenital hemolytic anemia caused by defect in the beta globin chain synthesis.[1] Beta thalassemia is considered to be the most common autosomal single gene disorder worldwide with a particularly high frequency in broad belt, extending from the Mediterranean basin through Middle East, India and South East Asia.[2] The mainstay in management in patients with Beta-Thalassemia is lifelong transfusion of RBCs and iron chelation.[3,4] The goal of long-term transfusion support is to maintain patient's hemoglobin at 9.5-10g/dl.

Although chronic blood transfusion is lifesaving, this is associated with the complications like erythrocyte alloimmunization and poses a major challenge for repeated transfusions in these patients. It increases the cost and complexity of cross-matching, shortens the RBC survival, delays the provision of safe blood and may accelerate the iron loading.[5] The factors for alloimmunization are complex and involve 3 main contributing elements: the RBC antigenic difference between donor and the recipient, the recipient immune status and immunomodulatory effect of the allogenic blood transfusion on the recipient's immune system.[6] Some alloantibodies are hemolytic and may cause hemolytic transfusion reactions which further limit the availability of safe blood for transfusion. Presence of hemolysis results in difficulty of cross- matching of blood. Erythrocyte autoantibodies appear less frequently, but they can result in difficulty in cross-matching of blood. Patients with autoantibodies may have a higher transfusion rate and often require immunosuppressive drugs, splenectomy or alternate treatment.[7,8] The development of RBC alloantibodies or autoantibodies against RBC antigens complicate RBC cross-matching, shortens in vivo survival of transfused cells and may accelerate tissue iron loading.[9]

The distribution of various blood group antigens varies amongst individuals in any given population.[10] Therefore, there is a variable

degree of disparity amongst the donors and the recipient regarding the group systems other than 'ABO' and Rh 'D', which are not tested before routine transfusion.

A wide discrepancy in the distribution of certain blood groups antigens and between donor and recipient can be responsible for a higher risk of alloimmunization in multiple transfused patients.[11] In eastern parts of India 5.6% of population have Beta- Thalassemia trait and 5% of population have HbE carrier state.[12] But there is lack of data on the incidence RBC alloimmunization in thalassemic patients as pretransfusion screening is not routinely performed.

Thus, this study will be conducted in Department of Immunohematology and Blood Transfusion to screen for alloimmunization and autoimmunization in transfusion dependent thalassemia major patients registered. So that adverse reactions like hemolytic transfusion reaction can be prevented and Blood Transfusion Services can be improved.

6.2 REVIEW OF LITERATURE

The transfusion of blood and blood components has become an important part in the management of patients in the era of modern medicine. So it is the responsibility of Blood Transfusion Services to ensure safe and quality blood products. Blood transfusion support is an important part in the management of patients with hematological disorders and malignancies. Many such patients require blood transfusion during illness or maybe lifetime.[13]

The discovery of blood group system by Karl Landsteiner in 1900 and development of Anti-Globulin test by Coombs, Mourant and Race in 1945 led to marked reduction in fatalities associated with blood transfusion and identification of various antibodies. Recently, 15 blood group antigens have been added to eight of the blood group system as recognized by International Society of Blood Transfusion making a total of 360 blood group antigens, of which 322 are clustered within 36 blood group system.[14]

Constant efforts are being made to ensure the safety of blood and its products. This has led to introduction of screening protocols for the detection of unexpected antibodies. The ultimate goal is to determine the exact specificity of the antibody and to provide blood that lacks the corresponding antigen to the patient.[15]

Alloimmunization against Red Blood Cells, Leucocytes and platelet antigens occurs due to genetic diversity of antigens between blood donors and recipients.[16] RBC alloimmunization is an immune response against foreign RBC antigens after sensitization due to blood transfusion and pregnancies. Clinically significant alloantibodies develop in 6-36% of multi-transfused patients and can pose major problems in the long term transfusion therapy.[17]

Alloimmunization further complicates the transfusion therapy due to difficulty in getting blood and delayed type of hemolytic transfusion reaction. It is one of the major concerns in the management of patient who require repeated blood transfusion as a life saving option. In patients affected with hemoglobinopathies, hematological diseases, various types of cancers, recipient of organ transplantation and

patients with renal failure the prevalence of alloimmunization has been reported to be up to 60%.[18]

Antibodies can either be alloreactive or autoreactive. Alloantibodies are produced after exposure to genetically different, or non-self, antigens, such as different RBC antigens after transfusion. Transfused components may elicit formation of alloantibodies against antigens (red cell, white cell and platelets) not present in the recipient. Autoantibodies are produced in response to self-antigens. They can cause reactions in the recipient if they they have specificity that is common to transfused blood.

A potentially serious problem for blood bankers is transfused patients who have alloantibodies that are no longer detectable in patient's plasma or serum. If these patients are transfused with immunizing antigen again, they will make a stronger immune response against those RBC antigens, which can cause severe and possibly fatal transfusion reaction. Factors influencing the rate of alloimmunization are antigenic differences, dose, frequency of transfusion, recipient's immune status, and immunogenicity of the donor HLA antigens.[19]

The recipient's serum or plasma should be tested for clinically significant unexpected alloantibodies. In general "clinically significant unexpected antibodies" refers to the antibodies that are reactive at 37'C or in the antihuman globulin and are known to have caused a transfusion reaction or unacceptably short survival of transfused antibodies[20].Reported alloimmunization rates ranged from 4% to 50% in thalassemia and were lower in more homogenous population[21].

In a study performed by Sylvia T Singer et al in 2000, clinical and transfusion records of 64 patients were analyzed 19 alloantibodies were identifies, 14 of them clinically significant. 3 patients developed 2 antibodies, and 3 antibodies were detected in 1 patient. Out of 64 patients 6 patients developed autoantibodies as determined by a persistent or transient positive DAT that ranged between +1 to +4 and was based on a maximum score of 4+. In 7 patients autoantibodies were associated with alloantibodies.

In another study performed by Khalid et al (in 2004) 75 cases of multiply transfused thalassemia major patients were investigated for development of alloantibodies against red cells by indirect antiglobulin test, using 3-red cell panel, and when required 11-red cell panel. Anti-red cell alloantibodies were detected in 17 (22.7%) patients. Anti-Kpa antibodies were the commonest, followed by Anti-e, anti-E and anti-K antibodies, respectively. Anti-k, -CW, -Fyb, -Kpb, -Rh 'D' and –c were detected in one patient each.

In a study carried out by Mohammed Hadi Sadeghian et al in 2009, 313 pateints were screened and 12 alloantibodies in 9 patients (2.87%) were against Rhesus (Rh) blood group antigens (D, C, E). Three patients developed 2 antibodies, and others had one antibody. The most common alloantibodies were Anti-D (88.88%) and followed by Anti-C and Anti-E. Higher frequency of alloimmunization was observed in female, Rh negative and splenectomized patient

In another study performed by HK Dhawan, Vijay Kumawat, Deepak Bansal[22], Satyam Arora[23] et al in 2014 study was carried out 319 transfusion dependent thalassemic patients, 18 patients developed alloantibodies. A total of 23 alloantibodies were identified in patient population. Out of 23 alloantibodies , 52.17% belonged to Rh blood group system(Anti-E=17%, Anti D=13%, Anti C=13%, Anti Cw =9%), 35 % belonged to Kell Blood group system, 9% of Kidd, 4% of Xg blood group system. 14 patients developed single antibody while 4 patients developed dual antibody.

Another study was performed Suvro et al in the year 2015. 500 thalassemia patients were reviewed. 28 cases were reported for alloimmunization Antibody c had the highest incidence(28.57%) followed by E(21.42%), Jkb (3.57), C (3.57%), D(3.57%). 5 patients had developed autoantibodies as determined by positive auto control on gel card(IgG+C3d) as weel as positive Direct Agglutination test.

In a study performed by Mohammed et al. in 2015 55 patients with beta thalassemia major were analyzed. 1 patient was tested positive for anti-kell.

6.3 AIMS AND OBJECTIVES OF THE STUDY

The aim of this study is

1. To study the frequency of RBC alloimmunization and autoimmunization among thalassemia patients.
2. To analyze the factors responsible for development of antibodies.

7. MATERIALS AND METHODS

7.1 Source of Data

This study will be conducted in Dayanand Medical College And Hospital, Ludhiana. This study will be conducted in beta thalassemia patients who have already been registered with the institute and are on regular blood transfusion.

Transfusion records will be examined for the details like age of the patient, age at diagnosis and initiation of transfusion therapy, total number of units transfused, type of blood transfused and transfusion interval and splenectomy status.

7.2 METHOD OF COLLECTION OF DATA

Patient's sample will be obtained in EDTA vial for ABO& Rh blood grouping and crossmatch from the thalassemia ward along with name and MRD NO. of the patient.

3ml of EDTA blood sample will be taken and centrifuged at 3500*g for 3 mins to obtain plasma for cross matching and antibody screening and red cell for detection of autoantibodies on gel card system. Prior to every transfusion plasma will be tested by using commercial 3 cell panel (Immucor and BioRad). All antibody screening positive sample will be evaluated to identify the antibody specificity. Antibody specificity detection will be performed if 3 cell panel positive using a commercial 11 cell identification panel (Diapanel, BioRad. Switzerland). Auto control will be performed in each case to identify autoantibodies. Autoantibodies will be detected by Direct Agglutination Test and Auto-control. Auto-control will be performed by incubating patient cell with plasma at 37°C for 15 minutes and then centrifuging for 10 mins on gel card containing polyspecific anti-human globulin (anti IgG+ C3d).

INCLUSION CRITERIA

1. All thalassemia patients registered with the hospital.

EXCLUSION CRITERIA

1. Alloimmunizaed Thalassemia major patients.

DURATION OF STUDY: 1 Year

OUTCOME: Alloimmune and autoimmune anti bodies in transfusion dependent thalassemia major patients.

STATISTICAL ANALYSIS: Analysis will be conducted using SPSS for windows (version 21) by making frequency distribution tables and identifying frequency of alloimmunization and autoimmunization as well as specificity of the particular antibodies. Comparisons of categorical data will be made using Pearson Chi Square test and Fisher's exact test. A p-value less than 0.05 will be considered significant.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

Not Applicable

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Yes

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PROFORMA

S. No

Name **Age**

Sex

Male

Female

Transgender

ABO & Rh

Marital status: Unmarried/Married

Locality: Urban/Rural

- **Region**

  Punjab

  Himachal Pradesh

  Rajasthan

  Jammu & Kashmir

  Haryana

  Others

- **Religion:** Hindu Muslim Sikh Others

Caste groups

Occupation

Family history

Years of transfusion

Total units transfused

Transfused requirement

Direct agglutination

Indirect Agglutination Test

Antibody Screening 3-cell Panel

Antibody Identification 11 cell Panel

Autocontrol

Splenectomy Status

Signature of Candidate **Signature of Supervisor**

INFORMED PATIENT CONSENT

Date: _____

I.....D/o, W/o, S/o..... .....am willing to participate in the study **"TO STUDY ALLOIMMUNIZATION AND AUTOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA MAJOR PATIENTS"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

.....
Signature of the Witness

Name:

.....
Signature of the patient

Signature of Candidate

Signature of Supervisor

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____

इस शोधकार्य **"TO STUDY ALLOIMMUNIZATION AND AUTOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA MAJOR PATIENTS"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

_____ _____

गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

_____

अन्वेषक के हस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

"TO STUDY ALLOIMMUNIZATION AND AUTOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA MAJOR PATIENTS" ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

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ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

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ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

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ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ